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AUS DER MEDIZINISCHEN ABTEILUNG DES STÄDTISCHEN MARIEN KRANKENHAUSES HELSINKI

KLINISCHE UNTERSUCHUNGEN ÜBER DIE ABSTUFUNG DER MUSKELTÄTIGKEIT

von

MARTTI HIRVONEN und M. A. RÄSÄNEN

(Eingegangen am 28. Mai 1952)

Der eine von uns (Hirvonen) hat früher in einer Arbeit zusammen mit Renqvist (Reenpää) und Uotila (1932) die elektrische Gesamtreizbarkeit von zwei Hinterbeinmuskeln des Frosches untersucht. Das Verhältnis zwischen der Maximal- und der Minimalrheobase dieser Muskeln wurde in 69 Versuchen bei 56 Fröschen bestimmt und dabei konnte festgestellt werden, dass das Verhältnis der Mittel- bzw. der Maximalrheobase zu der Minimalrheobase eine von der absoluten Höhe der Rheobasen unabhängige, ziemlich gute, bei den genannten Muskeln gleich grosse Konstante war.

M. gastrocnemius, der eine von den untersuchten Muskeln, ist ein recht starker Muskel, dessen durch Kondensatorentladungen hervorgerufene maximale Spannung in der Regel zwischen 300 und 450 g beträgt; M. peronaeus, der andere von den Muskeln dagegen ist ein verhältnismässig schwacher Muskel, und seine Maximalspannung steigt öfters nicht über 40 g, in vielen Fällen nicht einmal über 10 g. Bei der Schwellenzuckung entwickeln diese Muskeln dagegen eine so gut wie gleich grosse Schwellenspannung von etwa 1 g.

Die von einer Muskelkontraktion hervorgerufene Arbeitsleistung hängt ihrer Stärke nach einerseits von der Impulsfrequenz und andererseits von der Zahl der sich jeweils kontrahierenden motorischen Einheiten des Muskels ab. Obgleich die Impulsfrequenz erheblich variieren kann, liegt ja ohne weiteres auf der Hand, dass die Zahl der sich bei der Schwellenzuckung kontrahierenden motorischen Einheiten gering sein muss. Gleichfalls muss die Zahl der

sich bei der Maximalzuckung kontrahierenden motorischen Einheiten, unabhängig von der Impulsfrequenz, ziemlich gross sein.

Da die verschiedenen motorischen Einheiten der Muskeln auf jede elektrische Reizspannung mit wenigstens annähernd gleich starken Muskelspannungen antworten dürften, so müssen bei einem

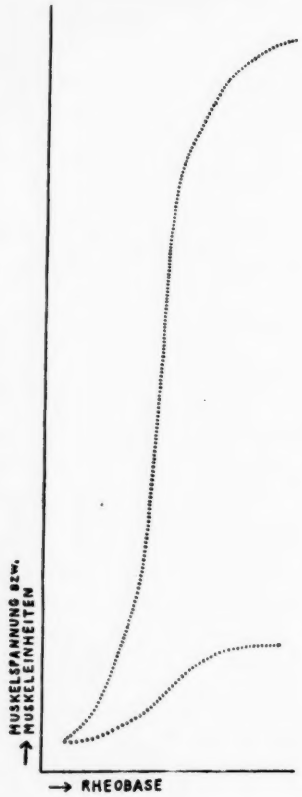

Fig. 1.

starken Muskel viel zahlreichere motorische Einheiten in Funktion treten, als bei einem schwachen Muskel, wenn die Reizspannung von dem dem Schwellenwert der Muskelspannung bis zu dem der Maximalspannung der Muskelspannung entsprechenden werte zunimmt.

Die Figur 1 soll diese Verhältnisse näher veranschaulichen. Diese Figur zeigt die Abhängigkeit der Muskelspannung eines M.

gastrocnemius und eines M. peronaeus von der Rheobase. (Hirvonen 1932) Die Kurve des M. gastrocnemius läuft ersichtlich viel höher als die des M. peronaeus.

Die S-Form der Kurve in Fig. 1 bedingt wiederum bei Annahme gleich grosser Spannungen der verschiedenen motorischen Einheiten, dass eine neue Einheit bei mittelgrosser Spannung des Muskels durch eine mehrfach kleinere Erhöhung der Reizspannung in Funktion treten muss, als bei einer kleinen oder einer grossen Spannung des Muskels. Die das Verhalten der verschiedenen aufeinander folgenden Einheiten des Muskels zu bezeichnenden Punkte der Verteilungskurve müssen also in der Richtung der Abszisse in der Mitte der Kurve mehr zusammengedrängt sein als an den beiden Enden der Kurve. Diese Eigenschaft der Kurve muss umso stärker hervortreten, je steiler die S-Kurve läuft, also je grösser die Maximalspannung des Muskels ist. Man soll also bei einem schwachen Muskel bei unveränderter Impulsfrequenz die Reizspannung beträchtlich mehr vergrössern als bei einem starken Muskel, um besonders bei mittelgrosser Spannung des Muskels die in der Reizbeziehung nächstfolgende motorische Einheit in Funktion zu bringen, m.a.W. um dieselbe Spannungszunahme in den Muskeln zu bewirken.

Wenn man nun eine ihrer Grösse nach genau bestimmte Muskelspannung erzeugen will, so folgt aus dem Obendargestellten, dass man die dazu erforderliche elecktrische Reizspannung bei einem grossen Muskel viel genauer als bei einem kleinen Muskel abstufen muss. In Übereinstimmung hiermit zeigte es sich auch in der früher erwähnten Arbeit, dass bei dem M. gastrocnemius, also bei einem grossen Muskel, bei mittlerer Arbeitsleistung desselben die Spannung des Muskels nur in relativ grossen Schritten durch Reizerhöhung zu vergrössern war, die motorischen Einheiten des Muskels also nie einzeln, sondern nur gruppenweise in Funktion zu bringen waren.

Wenn also ein einzeln stattfindendes Infunktiontreten der in elektrischer Reizbeziehung einander nahe stehender motorischer Einheiten eines grossen Muskels besonders bei einer mittelgrossen Arbeitsleistung desselben schwer oder unmöglich durch die Abstufung des elektrischen Reizes zu erzielen ist, liegt der Gedanke nahe, dass auch die genaue willkürliche Abstufung der Muskelspannung eines grossen Muskels schwerer oder eventuell bei mittelgrosser

Spannung desselben gar nicht zu erzielen wäre. Dagegen könnte man sich vorstellen, dass bei einem kleinen Muskel, dessen Spannung durch Abstufung des elektrischen Reizes viel genauer zu erzielen ist, analog auch die willkürliche zentralnervöse Steuerung sogar die kleinsten Spannungsänderungen, also die Feinregulierung der Spannungsleistungen bewältige.

Das eventuelle Vorhandensein eines solchen Unterschiedes in der willkürlichen zentralnervös bedingten Tätigkeitsart der grossen und kleinen Muskeln ist meines Erachtens am besten durch Vergleich der Tätigkeit von verschieden grossen Agonisten zu untersuchen.

Die gegenseitige Tätigkeit verschiedener Agonisten ist früher eingehend von Wachholder (1928) mit der Aktionsstrommethode untersucht worden. Diese Arbeit wird jedoch nicht näher referiert, weil die darin erhaltenen Resultate in keiner Beziehung zu dieser Arbeit stehen.

Die Möglichkeiten einer Abstufung des Innervationsvorganges und der Intensitätsvariationen zentripetal verlaufender Impulse sind von Adrian und Bronh (1929) und von Brücke (1929) untersucht worden. Sie folgerten, dass jede Abstufung in erster Linie dadurch zustande käme, dass die Zahl der jemals erregten Einheiten oder Elemente variiere. Dazu kommt als zweiter wichtiger Faktor noch die Variation der Frequenz der in einer nervösen Bahn aufeinander folgenden Erregungswellen.

FRAGESTELLUNG

Unsere Fragestellung gründet sich auf die Vermutung, dass bei willkürlicher Muskeltätigkeit die Arbeitsleistung einer zusammengesetzten Muskeltätigkeit mehrerer Agonisten haptsächlich von den grossen und starken Agonisten entwickelt, während dagegen die feinere Abstufung der Spannungen je nach dem beabsichtigten Zweck oder der Beanspruchung vorwiegend von den kleinen und schwachen Agonisten versorgt werde. Diese Fragestellung teilen wir noch folgendermassen ein.

1) Ist die Abstufungsfähigkeit einer willkürlichen Muskeltätigkeit schlechter bei mittelgrossen Spannungen als bei kleinen oder grossen Spannungen eines Muskels bzw. einer Muskelkombination? Die Genauigkeit der Abstufungsfähigkeit wird einfach durch

die Differenz der zu prüfenden und der vorangegebenen Muskelspannung bestimmt, wenn die Versuchsperson bemüht war, dieselben gleich gross zu gestalten. Diese Genauigkeit der Abstufungsfähigkeit kann die Abstufungsschwelle genannt werden.

2) Ist die Abstufungsfähigkeit eines kleinen und schwachen Muskels oder mehrerer kleinen und schwachen Muskeln besser, als diejenige eines grossen und starken Muskels oder mehrerer grossen und starken Muskeln? Ein grosser Muskel braucht nicht die Grösse der Spannungen ebenso genau wie ein kleiner Muskel abzustufen, damit sie beide eine gleich gute Abstufungsmöglichkeit in Bezug auf ihre maximalen Spannungen hätten, m.a.W., damit die zu vergleichenden Muskeln mit der gleichen Anzahl von Spannungen verschiedener Grösse zustande kommen könnten. Die Abstufungsfähigkeit in Bezug auf die maximale Muskelspannung kann relative Abstufungsfähigkeit genannt werden.

3) Die Abstufungsfähigkeit einer Patientin mit schwerer Muskelatrophie, diejenige eines Patienten mit Myotonia congenita und die einer Patientin mit arthritischen Gelenkveränderungen werden einer Untersuchung unterworfen.

METHODIK

Die Versuche wurden mit direkter Registrierung der Muskelspannungen ausgeführt. Figur 2 stellt den in dieser Versuchsanordnung benutzten isometrischen Myographen vor. In den Versuchen wurde die Tätigkeit der verschiedenen Flexoren des Zeige- und des Ringfingers untersucht. Diese Flexoren sind M. flexor digitorum sublimis, M. flexor digitorum profundus, Mm. lumbricales und Mm. interossei. In Bezug auf ihre Tätigkeit unterscheiden sich diese Muskeln darin, dass die kleinen Mm. lumbricales und Mm. interossei nur die erste Phalanx des Fingers, M. flexor digitorum profundus nur die dritte Phalanx des Fingers und M. flexor digitorum sublimis sowohl die zweite als die dritte Phalanx des Fingers beugen. (Duchenne 1885).

Um die Tätigkeit verschiedener Muskelkombinationen untersuchen zu können, wurde das distale oder das proximale interphalangeale Gelenk des zu untersuchenden Fingers je nach Bedarf mit den in Figur 2 dargestellten Fingerstützen unbeweglich gemacht. Zum Stützpunkt während der auszuführenden Flexionen, in denen die Versuchsperson den metallenen Knopf des Myographen herun-

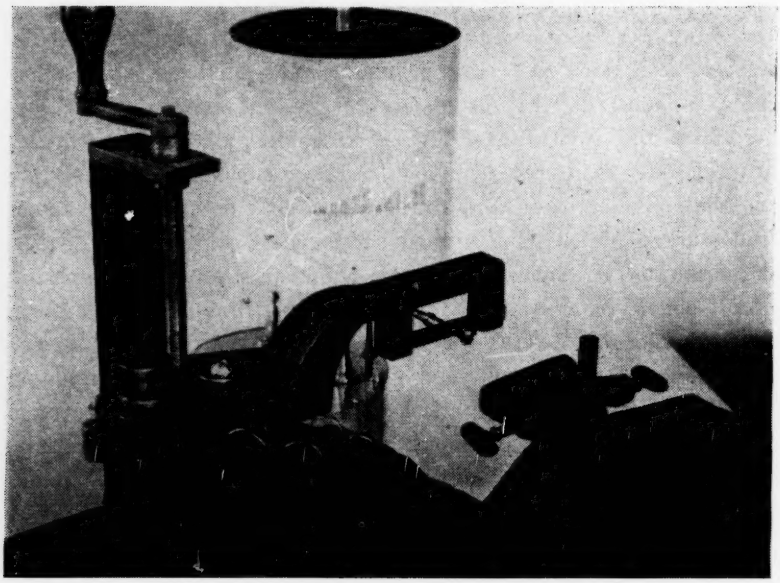

Fig. 2.

terdrückte, wurde je nach Bedarf entweder der Kopf des Metacarpalknochens oder der der ersten Phalanx gewählt. Bei dieser Versuchsanordnung gab es folgende Kombinationsmöglichkeiten zur Untersuchung der Tätigkeit der genannten Agonisten:

Das distale Gelenk	Das proximale Gelenk	Der Stützpunkt am Kopf	In der Funktion
frei	fixiert	der ersten Phalanx	M. flexor digit. profundus
frei	frei	der ersten Phalanx	Mm. flexor. digit. profundus und sublimis
fixiert	frei	der ersten Phalanx	Mm. flexor. digit. profundus und sublimis
frei	frei	des Metacarpalknochens	alle
fixiert	fixiert	des Metacarpalknochens	Mm. lumbricales und interossei

Der Gang der Untersuchung war wie folgt. Die Versuchsperson führte mit beiden zu untersuchenden Fingern und mit allen genannten Muskelkombinationen drei Serien von Flexionen aus. Die Serien waren von drei verschiedenen Grössenordnungen und bestanden aus 1) möglichst leichten, 2) mittelstarken und 3) ungefär maximalen Flexionen. Die Anzahl der Flexionen in jeder Serie war 60. Die Aufgabe der Versuchsperson in diesen Versuchen war, ohne mit dem Auge der Bewegung des Hebelarms des Myographen zu folgen, jede Flexion möglichst ebenso gross wie die vorhergehende zu gestalten.

Die Apparatur ist von Herrn Prof. Y. Reenpää zu unserer Verfügung überlassen und wir wollen ihm dafür unseren besten Dank aussprechen.

VERSUCHSERGEBNISSE

Versuche wurden mit 4 Personen ausgeführt: 1) mit einer muskel-, nerven- und gelenkgesunden Person, 2) mit einer Patientin, die eine hochgradige Inaktivitätsatrophie der Muskeln der linken Hand hatte, 3) mit einem an Myotonia congenita leidenden Patienten und 4) mit einer Patientin, die leichte polyarthritische Deformitäten an den Fingern hatte.

In der Tabelle 1 wird das Resultat von den Versuchen mit der muskel-, nerven- und gelenkgesunden Person dargestellt. Folgende Abkürzungen wurden gewählt: die in jeder Versuchsserie untersuchte Hand R oder L, der Finger II oder IV und die Muskelkombination p = M. flexor digit, profundus, s = M. flexor digit. sublimis, l = Mm. lumbricales und i = Mm. interossei. Von den 60 Flexionen jeder Versuchsserie ist zuerst der Mittelwert der Ausschläge des Myographen (AM) berechnet. Die folgende Kolonne der Tabelle gibt den Mittelwert der Abstufungsschwellen (Schw) an. Die in der letzten Kolonne dargestellte relative Abstufungsfähigkeit (RA) ist in Prozent umgerechnet.

Aus der Tabelle geht deutlich hervor, dass die Abstufungsfähigkeit am besten bei den kleinen Muskelspannungen und am schlechtesten bei den maximalen Muskelspannungen ist. Was wiederum die Abstufungsfähigkeit verschiedener Muskelkombinationen bei Muskelspannungen derselben Grössenordnung anbetrifft, ist sie einigermassen von dem Mittelwert der Ausschläge des Myographen,

TABELLE 1

RESULTATE DER VERSUCHE MIT EINER MUSKEL-, NERVEN- UND GELENK-GESUNDEN PERSON

	Kleine Muskelspannungen			Mittelstarke Muskelspannungen			Maximale Muskelspannungen		
	AM	Schw	RA	AM	Schw	RA	AM	Schw	RA
R II l+i	2.3	0.43	3.0	4.9	0.56	4.0	14.0	1.21	8.6
R II l+i+s+p	4.5	0.60	2.8	10.4	1.13	5.3	21.3	1.92	9.0
R II s+p (I)	4.0	0.90	4.7	4.5	0.90	4.7	19.3	1.63	8.4
R II s+p (II)	3.3	0.76	3.4	9.3	1.05	4.7	22.2	1.49	6.7
R II p	3.9	0.60	3.0	10.5	1.09	5.5	19.9	1.25	6.2
L II l+i	1.7	0.60	3.8	5.6	0.66	4.2	15.5	1.97	12.7
L II l+i+s+p	5.5	0.59	3.6	11.3	1.49	9.0	16.5	1.84	11.2
L II s+p (I)	4.4	0.79	5.4	7.2	1.30	8.9	14.7	2.04	13.9
L II s+p (II)	6.3	1.45	7.1	13.1	2.51	12.3	20.4	2.87	14.1
L II p	5.0	0.91	5.4	10.8	2.12	12.6	16.8	2.81	16.7
R IV l+i	3.7	0.45	2.9	6.3	0.94	6.1	15.3	1.31	8.6
R IV l+i+s+p	6.0	1.36	8.1	12.4	1.39	8.3	16.7	1.98	11.9
R IV s+p (I)	0.9	0.37	2.5	8.4	0.91	6.0	15.1	1.22	8.1
R IV s+p(II)	5.3	0.91	5.7	8.8	1.14	7.2	15.9	1.40	8.8
R IV p	0.5	0.14	1.0	4.3	1.00	7.0	14.3	1.42	9.9
L IV l+i	1.3	0.43	3.7	3.1	1.02	8.9	11.5	1.49	12.9
L IV l+i+s+p	1.9	0.68	3.6	7.0	0.86	5.9	14.5	2.02	13.9
L IV s+p (I)	3.9	0.74	4.3	10.1	1.26	7.3	17.3	2.18	12.6
L IV s+p (II)	2.6	0.63	3.8	6.0	1.03	6.1	16.7	1.69	10.1
L IV p	2.8	0.72	3.9	7.6	1.09	6.0	18.3	2.22	12.1

Erklärungen der Tabelle im Text

also auch von dem Mittelwert der Muskelkraft in den ausgeführten Flexionen abhängig. Wenn der Mittelwert der Muskelkraft irgend einer Muskelkombination, von der Regel abweichend, bis auf das Niveau des Mittelwertes der Muskelspannungen der folgenden Grössenordnung gestiegen ist, zeigen der Schwellenwert der Abstufung und die relative Abstufung auch dieser Grössenordnung entsprechende Werte.

Beim Vergleich der Resultate der rechten Hand mit denjenigen der linken Hand kann kein sicherer Unterschied wahrgenommen werden. In den maximalen Muskelspannungen zeigen die Versuche mit der linken Hand zwar etwas höhere Schwellenwerte der

TABELLE 2

RESULTATE DER VERSUCHE MIT EINER AN INAKTIVITÄTSATROPHIE DER MUSKELN DER LINKEN HAND LEIDENDEN PATIENTIN

	Kleine Muskelspannungen			Mittelstarke Muskelspannungen			Maximale Muskelspannungen		
	AM	Schw	RA	AM	Schw	RA	AM	Schw	RA
R II l+i	3.6	0.30	1.7	7.0	0.80	4.5	17.8	1.41	7.9
R II l+i+s+p	3.5	0.35	2.0	10.5	1.15	6.7	17.1	1.56	9.1
R II s+p (I)	4.3	0.46	2.8	9.9	0.90	5.5	16.4	1.20	7.3
R II s+p (II)	4.8	0.60	4.1	8.4	0.80	5.5	14.6	1.40	9.6
R II p	4.2	0.40	2.5	14.6	1.00	5.6	16.0	1.40	8.7
L II l+i	2.6	0.60	6.7	6.3	0.95	10.7	8.9	0.65	7.3
L II l+i+s+p	4.3	0.57	5.4	6.3	0.84	7.9	10.6	1.74	16.3
L II s+p (I)	2.1	0.73	5.5	7.9	1.03	7.8	13.2	1.57	11.9
L II s+p (II)	5.0	0.70	4.6	10.3	1.17	7.6	15.3	1.43	9.3
L II p	6.9	1.02	6.1	10.1	0.88	5.3	16.7	1.95	11.7
R IV l+i	3.3	0.42	2.7	11.6	1.38	8.8	15.6	1.32	8.5
R IV l+i+s+p	2.0	0.67	4.4	8.3	1.29	8.5	15.1	2.14	14.2
R IV s+p (I)	1.5	0.33	2.5	7.1	0.66	5.0	13.3	1.55	11.7
R IV s+p (II)	4.3	0.88	5.9	11.9	1.10	7.4	14.8	1.37	9.3
R IV p	3.6	0.82	4.1	9.3	0.84	4.2	19.9	1.69	8.5
L IV l+i	3.5	0.51	4.4	7.6	1.08	9.3	11.6	1.29	11.1
L IV l+i+s+p	4.8	0.86	8.1	7.8	1.12	10.6	10.6	1.41	13.3
L IV s+p (I)	4.9	1.00	8.6	7.7	1.75	15.1	11.6	1.14	9.8
L IV s+p (II)	5.0	0.92	6.8	7.9	1.02	7.6	13.5	1.06	7.9
L IV p	3.3	0.62	5.2	7.2	0.89	7.4	12.0	1.15	9.6

Abstufung und bei ungefär gleich starken Flexionen auch etwas höhere Prozentzahlen der relativen Abstufung, aber diese Veränderungen sind zu klein um beweisend zu sein.

Die zweite Versuchsperson hatte nach einem Schaftbruch des linken Radius eine schwere Muskelatrophie der Muskeln des Antibrachiums und der Hand. Die Muskeln der rechten Hand waren gesund. Tabelle 2 stellt die Resultate von den Versuchen mit dieser Patientin dar.

Die mit der gesunden rechten Hand dieser Patientin erhaltenen Resultate unterscheiden sich in keiner Weise von denjenigen unserer ersten Versuchsperson. Die maximalen Muskelspannungen der atrophischen linken Hand waren natürlich in manchen Muskel-

TABELLE 3

RESULTATE DER VERSUCHE MIT EINEM AN MYOTONIA CONGENITA LEIDENDEN PATIENTEN

	Kleine Muskelspannungen			Mittelstarke Muskelspannungen			Maximale Muskelspannungen		
	AM	Schw	RA	AM	Schw	RA	AM	Schw	RA
R II l+i	4.2	0.47	2.7	7.2	0.96	5.6	17.2	1.31	8.2
R II l+i+s+p	3.0	0.57	5.1	6.7	0.87	7.8	11.2	1.08	9.7
R II s+p (I)	2.5	0.52	4.8	5.9	0.58	5.3	10.9	0.64	5.9
R II s+p (II)	2.7	0.62	3.6	8.0	0.80	4.6	17.2	1.16	6.7
R II p	4.2	0.42	3.2	9.7	1.00	7.6	13.2	1.10	8;3
R IV l+i	3.1	0.59	5.2	6.8	0.81	7.1	11.3	1.75	15.4
R IV l+i+s+p	2.7	0.61	6.5	6.7	0.91	9.7	9.4	1.36	14.4
R IV s+p (I)	2.4	0.57	3.8	12.2	0.94	6.3	15.0	1.70	11.4
R IV s+p (II)	2.8	0.62	4.2	8.7	0.87	5.9	14.7	1.39	9.4
R IV p	3.1	0.75	6.5	8.2	1.12	9.7	11.5	1.38	11.1

kombinationen bedeutend kleiner als diejenigen der rechten Hand. Damit übereinstimmend stiegen auch die relativen Abstufungen der atrophischen Hand oft höher als die jenigen der gesunden Hand. In den Abstufungsschwellen beider Hände war dagegen kein wahrnehmbarer Unterschied vorhanden.

Den an Myotonia congenita leidenden Patienten hat Herr Professor M. Kaila für diese Untersuchung überlassen und wir sprechen ihm dafür unseren besten Dank aus.

Von diesem Patienten wurde nur die rechte Hand untersucht und das Resultat wird in Tabelle 3 dargestellt.

Wie aus der Tabelle ersichtlich, unterscheiden sich weder die Abstufungsschwelle noch die relative Abstufung in diesem Fall von den mit der muskel-, nerven-, und gelenkgesunden Versuchsperson gewonnenen Resultaten.

Tabelle 4 zeigt die Resultate von den Versuchen mit der an Polyarthritis der Finger leidenden Patientin. Die Mittelwerte der Ausschläge des Myographen sind besonders in den maximalen Muskelspannungen, in geringerem Masse auch in den mittelstarken Muskelspannungen kleiner als in den früheren Fällen. Die Schwellenwerte der Abstufung sind dagegen kaum verkleinert. Damit übereinstimmend steigen die relativen Abstufungen oft höher als in den früheren Fällen.

TABELLE 4

RESULTATE DER VERSUCHE MIT EINER AN POLYARTHRITIS DER FINGER LEIDENDEN PATIENTIN

	Kleine Muskelspannungen			Mittelstarke Muskelspannungen			Maximale Muskelspannungen		
	AM	Schw	RA	AM	Schw	RA	AM	Schw	RA
R II l+i	2.7	0.39	3.6	4.8	0.61	5.6	10.8	0.93	8.6
R II l+i+s+p	3.8	0.63	4.6	6.2	1.26	9.3	13.6	1.70	12.5
R II s+p (I)	3.0	0.55	5.1	3.7	0.58	9.7	6.0	0.82	13.7
R II s+p (II)	1.7	0.52	5.7	4.1	0.72	7.8	9.2	1.06	11.5
R II p	2.8	0.61	13.1	2.8	0.41	8.8	4.6	0.79	17.0
R IV l+i	1.5	0.44	4.4	4.2	0.72	7.2	10.0	1.73	17.3
R IV l+i+s+p	2.6	0.57	7.2	5.9	0.80	10.1	8.0	1.06	13.3
R IV s+p (I)	3.3	0.70	11.6	3.9	0.78	13.0	6.0	0.82	13.6
R IV s+p (II)	3.7	0.67	10.2	5.1	0.75	11.4	6.6	1.09	16.6
R IV p	2.2	0.66	6.4	4.2	0.71	6.9	10.3	0.78	7.5

BESPRECHUNG DER ERGEBNISSE

Die in der Fragestellung ausgesprochene Vermutung, dass die Abstufungsfähigkeit einer willkürlichen Muskeltätigkeit vielleicht schlechter bei mittelgrossen als bei kleinen oder grossen Spannungen des Muskels bzw. der Muskelkombination sei, erwies sich somit als nicht stichhaltig. Der Schwellenwert der Abstufung war im Gegenteil von der Muskelkombination vollkommen unabhängig und wuchs in jeder Muskelkombination mit der Muskelspannung vom kleinsten Wert bei den kleinen Muskelspannungen zum grössten Wert bei den maximalen Spannungen.

Dieser deutliche Unterschied zwischen der Tätigkeitsart des mit Kondensatorentladungen erregten Froschmuskels und der des unter willkürlicher zentralnervöser Steuerung arbeitenden Menschenmuskels findet wohl seine Erklärung in der unphysiologischen Erregungsweise des Froschmuskels.

Der vorteilhafte Hebelarm hat zur Folge, dass die Muskelleistungen der ihrem Bau nach kleinen Muskeln in den meisten Fällen nur unbedeutend kleiner als diejenigen der ihrem Bau nach grösseren Muskeln sind. Deswegen kommen in der relativen Abstufung nur wenige unregelmässige Unterschiede zwischen den mit

verschiedenen Muskelkombinationen gewonnenen Resultaten zum Vorschein. Auch die Vermutung, dass die Abstufungsfähigkeit eines kleinen oder mehrerer kleinen Muskeln, unabhängig von der Muskelspannung, besser als diejenige eines starken Muskels oder mehrerer starken Muskeln wäre, hat sich also als nicht stichhaltig erwiesen.

Die im Zusammenhang mit der Muskelatrophie beobachteten Abweichungen beschränkten sich auf die Verkleinerung der maximalen Muskelspannung und auf die da von abhängigen Veränderungen. Bei der Myotonia congenita waren die Resultate unverändert. Polyarthritische Deformitäten an den Fingern verursachten auch Verkleinerung der maximalen Muskelspannung und davon direkt abhängige Veränderungen der Abstufung, wie die Muskelatrophie.

Die gewonnenen Resultate scheinen für die Vermutung zu sprechen, dass die bei elektrischer Erregung des Froschmuskels beobachteten Schwierigkeiten in der Abstufung mittelstarker Muskelspannungen wahrscheinlich von der unphysiologischen Erregungsweise abhängen und dass die Abstufung der Muskelspannung bei willkürlicher zentralnervöser Steuerung bei allen Muskeln von derselben Genauigkeit, und nur von der Grösse der ausgeführten Muskelspannung abhängig sei.

ZUSAMMENFASSUNG

Die Abstufungsfähigkeit der Muskelspannung der verschiedenen Flexoren der Finger unter willkürlicher Muskelarbeit und in verschiedenen Muskelkombinationen wurde mit direkter Bestimmungsmethode der Muskeltätigkeit mittels eines Myographen bei einer gesunden Versuchsperson, bei einer Patientin mit Muskelatrophie der Finger, bei einem Patienten mit Myotonia congenita und bei einer Patientin mit arthritischen Deformitäten der Finger untersucht. Folgende Schlüsse konnten daraus gezogen werden.

Der mittlere Schwellenwert der Abstufung, d.h. der Mittelwert der Differenzen nacheinander folgender Flexionen in einer Versuchsserie, wenn die Versuchsperson sie gleich gross zu machen versucht, erwies sich als von der Muskelkombination unabhängig und wuchs in jeder Muskelkombination mit der Muskelspannung vom kleinsten Wert bei den kleinen Muskelspannungen zum grössten Wert bei den maximalen Spannungen.

Die relative, d.h. auf die maximale Spannung der Muskelkombination bezogene Abstufung wuchs auf dieselbe Weise mit der Muskelspannung und wies nur unregelmässige Unterschiede zwischen den mit verschiedenen Muskelkombinationen gewonnenen Resultaten auf.

Im Zusammenhang mit der Muskelatrophie oder mit polyarthritischen Deformitäten an den Fingern kamen nur auf die Verkleinerung der maximalen Muskelspannung zurückzuführende Veränderungen der Abstufung zum Vorschein. Die Abstufungsfähigkeit des an Myotonia congenita leidenden Patienten war unverändert.

LITERATURVERZEICHNIS

1. Adrian, E. D., und D. W. Bronh: J. Physiol. 1929:*67:*119.
2. Brücke, E. Th.: Handbuch d. norm. u. path. Physiol. 1929:*9:*23.
3. Duchenne, G. B.: Physiologie der Bewegungen. — Cassel und Berlin. 1885.
4. Hirvonen, M.: Skand. Arch. Physiol. 1932:*64:*1.
5. Renqvist, Y., Hirvonen, M., und U. Uotila: Skand. Arch. Physiol. Physiol. 1932:*65:*60.
6. Wachholder, K.: Pflügers Arch. ges. Physiol. 1923:*119:*625. Ibid. 1925: *209:*218. Ibid. 1925:*210:*661. Ibid. 1926:*212:*666. Ergebn. d. Physiol. 1928:*26:*636.

FROM THE SURGICAL CLINIC, AND FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF TURKU

INFLUENCE OF ETHER ANESTHESIA ON THE WASSERMANN, CHOLESTEROL-WASSERMANN, AND KAHN REACTIONS

by

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In the Surgical Department of the District Hospital, Turku, a number of positive syphilitic reactions were observed in 1948, a fact which to many patients was a very unpleasant surprise since most of them had no previous knowledge that they had been infected. It is not unknown that ether anesthesia may cause non-specific positive Wassermann reactions (1, 2, 3, 4, 5, 6, 11, 18), and we therefore felt it indicated to investigate by means of random tests the frequency of non-specificity possibly caused by anesthesia. Cases due to accidents, in particular, are often operated on immediately upon admission, and routine tests for syphilis are not made until the following day, i.e. after operation. The blood in these cases might therefore be affected by the anesthesia.

In the literature the pseudo-positive results are generally divided into two groups:

1) technical and recording errors,

2) biologic non-specific positive reactions due to some disease other than syphilis (11, 21). Technical errors may be caused by bacteria that contaminate the blood when the sample is taken and by syringes and test tubes contaminated with sodium, alcohol, salvarsan, or other substances (15). Positive reactions may be obtained in as many as a hundred cases per cent in yaws, for

instance, which are caused by a spirochete, *Treponema pertenue* (21). Even the blood serum of normal people may be anomalous and give a positive reaction (11).

From the literature on this subject I have collected a number of different diseases and intoxications which may cause a non-specific positive syphilis reaction (1, 4, 5, 7, 9, 11, 20, 21, 23). I have divided these conditions into two groups 1) those that principally occur in our surgical cases and 2) those occurring in other series.

1)	2)
Empyema	Framboesia
Pneumonia	Malaria
Bronchitis	Leprosy
Pulmonary tuberculosis	Exanthematous typhus
Abscesses	Nephropathy
Sepsis	Scarlatina
Otitis media	Measles
Sinuitis max.	Epidemic parotitis
Pregnancy	Tonsillitis
Diabetes	Ulcus molle
Gonorrhea	Psoriasis
Malignant growths	Mycosis fungoides
Serious fractures	Herpes genitalis
Serum therapy	Pernicious anemia
Arthritis	Leukemia
Acute poliomyelitis	Multiple sclerosis
Anesthesia	Recurring fever
	Endocarditis lenta
	Lymphogranulomatosis
	Avitaminosis

This miscellanous collection of diseases indicates that a positive reaction for syphilis does not always indicate syphilis. In dubious cases one positive serologic result is not sufficient; the test must be repeated and in evaluating the result the clinical features must also be considered (11). If a positive reaction obtained during the course of some of the above-mentioned diseases changes into a negative one when the patient is recovering or has recovered, it may be that the first reaction was non-specific (5, 7). Since, how-

ever, the percentage error is small, the value of serologic reactions for syphilis is not reduced. Experience has shown that the Kahn reaction does not give false positive results in more than 1 per cent and the Wassermann reaction in hardly ½ per cent of cases (19). In 1937 Sievers (16) obtained non-specific definitely positive results in Wassermann tests in 0.57 per cent, in Cholesterol-Wassermann tests in 0.78 per cent, and in Kahn tests in 1.48 per cent of cases. The corresponding figures reported by Anttonen (1) [917 cases], are considerably higher: Wassermann 3 per cent, uncertain 1 per cent, cholesterol-Wassermann 4 per cent, uncertain 1 per cent, Kahn 6 per cent, uncertain 1 per cent. The following authorities have also dealt with the problem of non-specificity: Forsman (5) reported 10 non-specific reactions in 7,711 cases, and of these 3 were cases with some affection of the lungs. Turunen (20) investigated 2,390 cases of pregnancy and found 0.75 non-specific reactions per cent for syphilis. Honkanen's series (8) comprised 611 cases of phthisis, in which he obtained 0.7 per cent of non-specific Kahn reactions. Halonen (7) reported 3 cases of pulmonary infiltration in which positive reactions for syphilis were obtained during the course of the disease, these reactions changing to negative during convalescence. Penttinen's (10) thesis is based upon 18,095 cases of pregnancy in which non-specific Wasserman reactions were obtained in 0.03 per cent of cases, non-specific Kahn reactions in 0.4 per cent, and the two simultaneously in 0.01 per cent of cases.

Wolfsohn (22) was the first to investigate the serum of 50 patients anesthetized with ether, and in 11 cases he obtained a positive reaction which, in a few days, changed into a negative one. Wolfsohn used a mixed anesthesia of veronal, ether, and morphia. Reicher (12) twice established a positive Wassermann reaction after ether-chloral hydrate anesthesia. Rosenthal (14) made the same observation without specifying the nature of the anesthesia. Boas (2) investigated 60 cases anesthetized with chloroform and obtained 3 positive reactions which, in a week, changed into negative. Paraldehyde and amylene nitrate (2, 3) may also cause a positive Wassermann reaction. Sonntag (17) investigated 100 anesthetized cases without obtaining a single positive reaction. On the basis of these investigations Boas (2) draws the following conclusion: »Sera von narkositierten Patienten geben ab und zu

positive Wassermannsche Reaktion und dürfen für diese Untersuchung nicht verwendet werden». Stokes (18) declares that ether anesthesia, alcohol, and paraldehyde may give non-specific reactions for syphilis in abo t 20 per cent of cases. Gross (6) examined the blood of 30 patients anesthetized with ether and obtained a mild positive reaction in two cases. In these cases the duration of the anesthesia was two hours. A large quantity of alcohol may momentarily abolish a positive reaction for syphilis (2, 3, 6).

MATERIAL

The material dealt with in this article is a random series of 104 surgical cases. The patients had been subjected to scopolamine-morphia-ether anesthesia. Of these 53 were women and 51 men. A rough division according to the clinical diagnosis is as follows: 8 cases of cancer, 39 cases of inflammation, such as appendicitis, splenitis, abscesses, etc., and 57 so-called pure surgical cases, such as hernias, fractures, etc. The past history contained congenital syphilis in two cases, in both of which the patient had received treatment in due time. The serologic reactions for syphilis remained unchanged after anesthesia and during the course of the disease in one case, whereas changes were observed in the serologic reactions of the other case. One hitherto unnoticed case that might have been syphilis, was found. All serologic tests were made in the University Institute of Serology and Bacteriology, Turku, and as basic reactions the old approved methods, the Wassermann, the cholesterol-Wassermann, and the Kahn reactions were used.

Blood tests were made as follows:

Before Anesthesia	During Anesthesia	1 Day after Anesthesia	Total
49	49		49
30		30	30
6	6	6	6
	11		11
		3	3
	5	5	5
85	71	44	104

Control tests before anesthesia were made in 85 cases. 71 blood tests were made during anesthesia and 44 one day after it. Any influence of ether anesthesia could be expected therefore to appear in 115 examinations, but a positive reaction was obtained in two cases only, which are reported in greater detail.

Case 1. — Record No. 331/49. A.A.V.J., aged 28 years, welder, was treated in the hospital from Jan. 14, 1949, to Jan. 22, 1949, for concussion of the brain and seropositive syphilis. The patient denied venereal infection. He had generally been healthy. On Jan. 1, 1949, the patient was the victim of a traffic accident and had concussion of the brain. Routine blood test was done on Jan. 14, 1949, and the result was Wassermann negative, cholesterol-Wassermann positive, Kahn positive. When the patient was told of this result of the blood test he did not consider infection impossible although he had had no symptoms. The patient agreed to undergo ether anesthesia for »a more thorough examination of the blood test» and the results were as follows:

Jan. 18	under anesthesia	W.r.	±	Chol.W.r.	+	Kahn +
Jan. 21		»	—	»	+	» +
Jan. 22		»	+	»	+	» +
Febr. 5		»	—	»	+	» ±

It appears from this investigation that the Wassermann values fluctuate on the border between positive and negative. The result of the blood test made during anesthesia, Wassermann plus-minus, hardly depends on the anesthesia since on Jan. 22 a positive Wassermann reaction was obtained.

Case 2. — Record No. 2092/48. H.R.S., aged 22 years, a sailor's wife, treated in the hospital from May 17, 1948, to June 1, 1948, for acute gangrenous perforating appendicitis and diffuse peritonitis. She had had congenital syphilis. For two days she had had typical symptoms of appendicitis. Investigation revealed general peritonitis as a result of a perflrated appendix and operation was therefore immediately performed (appendicectomy and canalization of the abdominal cavity). During the operation it was found that the patient had Hutchinson's teeth; a blood test was therefore done during the anesthesia and the following result was obtained:

May 17	during operation	W.r.	±,	chol.W.r.	±	Kahn +		
May 18		»	—	»	+	» —		
May 20		»	—	»	—?	» —		
May 26	cerebrospinal fluid examination	W.r.	0.5 cc	—,	chol.W.r.	0.5 —		
			1	—		1 —		
May 27		W.r.	—,	chol.W.r.	—	Kahn —		

This was a case of congenital syphilis, treated until cured. Before the operation the patient's temperature was 38.5°. The result of the blood test done during operation was partly positive, which might depend either on the inflammation or on the anesthesia. On the next day the temperature was still 38.4° and despite this only the most sensitive reaction, the cholesterol-Wassermann, remained positive, though at that time the inflammatory process had scarcely been cured. On the third postoperative day, May 20, the patient still had a temperature of 37.6° and only the cholesterol-Wassermann gave an uncertain positive result. On May 27 the examination gave wholly negative results although there was still suppuration of the abdominal wall on May 29. It is impossible to state with certainty the reason for the temporary positivity, but it seems that the ether anesthesia together with the inflammation provoked the old congenital syphilis to react positively. Rein and Elsberg (13) write as follows on the duration of false positive Wassermann reactions: »From the studies at the Army Medical School, it seems that in most instances the maximum titer of false positive reactions is obtained between the tenth and fourteenth day following the onset of the febrile disease or following vaccination. The length of time an individual continued to show false positive reactions seemed to depend on the degree of positivity reached on the tenth to the fourteenth day.» This statement might support the assumption that the positive reactions for syphilis were caused by the anesthesia rather than by the temporary inflammation that had lasted but a couple of days. It cannot be analysed, nor ascertained with certainty whether the anesthesia, the inflammation, or both together caused the non-specific temporary reactions for syphilis.

On the basis of the present investigation the following conclusions might be drawn:

1) ether anesthesia did not cause non-specific Wassermann reactions in patients who did not suffer from syphilis.

2) It is possible that ether anesthesia may later cause reactions for a treated seronegative syphilis to appear as positive.

SUMMARY

The number of cases investigated was 104; blood tests were made during anesthesia and one day after it. Positive results were obtained in two cases only. The reactions of one case fluctuated on the border between positive and negative and the influence of ether anesthesia could not therefore be shown. The second was one of treated congenital syphilis operated on for appendicitis and peritonitis. During the anesthesia the reactions Wassermann plus-minus, cholesterol-Wassermann plus-minus, and Kahn positive were obtained. On the following day only cholesterol-Wassermann was positive. Later the reactions became quite negative. In this case the ether anesthesia together with the inflammation may have caused positive reactions for syphilis.

REFERENCES

1. Anttonen, V. M.: Über die Verstärkung der Wassermannschen Reaktion durch Zusatz verschiedener Cholesterine zum Rinderherzantigen und über die unspezifischen serologischen Luesreaktionen. — Academic Diss., Helsinki 1945.
2. Boas, H.: Die Wassermannsche Reaktion mit besonderer Berücksichtigung ihrer klinischen Verwertbarkeit. — S. Karger Berlin, 1911 and 1922.
3. Bruck, C.: Handbuch der Serodiagnose der Syphilis Part 2, Julius Springer, Berlin, 1924.
4. Eagle, H.: The Laboratory Diagnosis of Syphilis. — The C. D. Mosby & C:o, St. Louis, 1937.
5. Forsman, J.: Duodecim Acta A. 1932:*15:*1.
6. Gross, H.: Ztschr. f. Haut- und Geschlecht. 1949:*7:*223.
7. Halonen, P.: Duodecim 1945:*61:*398.
8. Honkanen, A.: Duodecim Acta A. 1936:*19:*1.
9. Kolle-Hetsch: Die Experimentelle Bakteriologie und die Infektionskrankheiten. Part 2, Urban & Schwarzenberg, Berlin, 1919.
10. Penttinen, K.: On the Wassermann and Kahn Reactions during Pregnancy. — Academic Diss., Helsinki 1946.
11. Penttinen, K.: Duodecim 1946:*62:*672.
12. Reicher, K.: D.m.W. 1910:*13:*617.
13. Rein, C. R., and Elsberg, E. S.: Am. J. Clin. Path. 1944:*14:*461.
14. Rosenthal, O.: B.k.W. 1910:*26:*1251.
15. Sievers, O. R. H.: Finska läk. sällsk. handl. 1936:*9:*820.

16. SIEVERS, O. R. H.: Finska läk. sällsk. handl. 1937:*80:*534.
17. SONNTAG: Cited in BOAS.
18. STOKES, J. H.: Modern Clinical Syphilology. — Saunders Company, Philadelphia and London, 1945.
19. STRENG, SIEVERS, and VUORI: Acta Soc. Med. Fennicae. — Duodecim Ser. A. 1933:*16:*F.L.
20. TURUNEN, A.: Duodecim 1935:*51:*831.
21. VOGELSANG, M.: Nord. med. 1945:*28:*2542.
22. WOLFSOHN, G.: D.m.W. 1909:*11:*505.
23. ZIELER, K.: Lehrbuch und Atlas der Haut- und Geschlechtskrankheiten. Part 2. — Urban & Schwarzenberg, Berlin and Wien, 1937.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY AND FROM THE SURGICAL CLINIC, UNIVERSITY OF TURKU

PATHOGENIC LACTOBACILLUS

by

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Filamentous micro-organisms, varying in size, occur frequently in the normal flora of the mouth and pharynx. Some of them, such as actinomyces, fusobacteria, bacteroides, and certain spirochaetes, are pathogenic, and from the alimentary canal or the respiratory passages may give rise to series diseases. Others, such as lactobacilli, leptotrichia, and many corynebacteria, are usually considered apathogenic. Lactobacilli in particular have been regarded as completely harmless. But, contradicting this theory, in a case treated at the Surgical Clinic, University of Turku, the serious malady from which the patient suffered could be traced to a species of lactobacilli; so I find myself justified in publishing this case and the bacteriological studies made in connection with it.

CLINICAL FEATURES OF THE CASE AND ITS TREATMENT

A.H., a farmer's daughter aged 18, had suffered from diabetes for 2 years. One week before admission to the hospital she developed a cough, which grew rapidly worse. After a few days she had a high temperature, and there was abundant, light sputum. When brought to the hospital on Oct. 13, 1948, the patient was unconscious and feverish. She was found to be in a state of diabetic coma (blood sugar being 0.321 and urinary sugar 8 per cent), which was controlled by insulin. There was abundant sputum, purulent, but not foul-smelling. Clinical and X-ray studies revealed a typical pulmonary abscess in the central part of the right lung. Severe leukocytosis (L 18920) was disclosed at the blood count, and the SR was 83 mm/1 hr.

Brownish-grey, non-smelling pus was obtained in a puncture of the lung abscess in which there were small white granules visible to the eye. The smear from the pus showed a great number of Gram-positive filamentous rods. This bacterium, which later grew on cultures, remained the only micro-organism in the abscess for the next 6 weeks. The same bacterium was also isolated several time from the patient's sputum. On the other hand, it did not occur in the samples taken from the carious teeth, tonsils, and the nasal mucous membrane. The staining for tubercle bacilli in the sputum was negative on 7 different days. Tubercle bacilli cultures from the sputum and stomach irrigation were likewise negative.

The patient was supposed to be suffering from pulmonary actinomycosis, which was treated by puncturing and evacuating the abscess and by antibiotics administered locally and generally. In choosing the antibiotics and calculating the dosages we profited by the studies made on the resistance of the isolated bacterium and the serum tests, to which I shall return later. First penicillin was used and later streptomycin and Elkosin, too, which was the only sulfonamide preparation the patient tolerated.

Treatment by punctures and antibiotics alone failed to cure the patient, for she still had a temperature and produced sputum in the 5th week, and bronchoscopy (Prof. Siirala) revealed an abundant flow of pus from the lung abscess into the bronchus of the right upper lobe. The abscess was canalized by operating in two stages (1. Resectio costae III et ablatio pleurae parietalis l.dx. 2. Canalisatio abscessus pulmonis l.dx. — Prof. Klossner). In the histologic examination the specimen taken from the abscess wall at operation was found to contain necrotic lung tissue infiltrated by pus cells, but no ray fungi (Pathological diagnosis: Necrosis et abscessus pulmonis. — Prof. Järvi). Bacteriological studies showed that the filamentous bacterium was still the only bacterium in the abscess but that it had decreased in number.

After the canalization of the abscess the patient's temperature fell to normal within a week and both the expectorations and the flow of pus from the drain became gradually thinner and ceased. At the same time the filamentous bacterium disappeared from the sputum and from the drained discharge. Other bacteria appeared at this stage in the discharge, namely *Staphyloc. aureus* and the apathogenic species of neisseria and corynebacteria.

The patient was discharged from the hospital 7 weeks after the operation (17. 1. 49) as convalescent. On leaving for home and in the follow-up made 2½ months later she was without symptoms except for a slight dullness in the right lung, which appeared as a densification in the roentgenogram.

In the follow-up inquires the patient was found to have been in good health for more than a year but had then developed pulmonary tuberculosis from which she died at home 23. II. 51 (she was under treatment at the Sanatorium of Finland Proper from 26. VI. 1950 to 20. I. 1951 the diagnosis being Tub. pulm. amb. gr. III A. Diabetes mellitus. Haemoptoe).

BACTERIOLOGICAL STUDIES

In the case described above the micro-organism found in the lung abscess occurred in the native preparations in the form of Gram-positive rods which were gently sinuous and filamentous, without ramifications (Fig. 1). The granules in the pus obtained from the abscess were found to consist of leukocytes and our bacterium, which was not in a radial arrangement like ray fungi. The micro-organism grew slowly in primary cultures and definite media were necessary for any growth at all. It did not usually grow in the absence of glucose or serum. From the very start the bacterium throve best under anaerobic conditions, but soon became used to oxygen. Boiled liver broth was a good first medium. CO_2 stimulated the growth of the bacterium.

In the different cultures, both aerobically and anaerobically, the bacterium grew as follows[1]: On the blood plate, as well as on Zeissler's glucose blood agar plate, small, round S-colonies appeared after 2—4 days. In 2—3 days they grew to between 1 and 3 mm in diameter and changed into R-colonies with uneven edges (Fig. 2). A medium-strong α-haemolysis always developed in them. 5—7 days later the colonies formed some brownish-yellow pigment. The bacterium grew more slowly on glucose agar, serum agar, and Löwenstein's and Löffler's media, and the colonies remained smaller in diameter. On the »Difco» medium also, in spite of 7 days' incubation under anaerobic conditions, the colonies were similar. The bacterium did not grow at all on an ordinary agar plate, or on the potate, or on Sabouraud's, Raulin's, and paraffin media. In a semi-fluid glucose deep agar tube it grew well. Vague diffuse obscurity developed in the liver broth after 1—2 days, settling down later on the tube bottom. In peptone water there was very slow growth at the bottom of the tube.

In plate cultures small S-colonies, which showed no changes, large R-colonies and their intermediate forms could occur simultaneously. In new cultures both S- and R-colonies grew again from both types of colonies — a dissociation phenomenon.

In sub-cultures, especially in fluid media, the bacterium often became Gram-negative. Its morphology varied greatly, depending

[1] The methods of culture and media used were described in more detail in an earlier study of mine (13, p. 37—38).

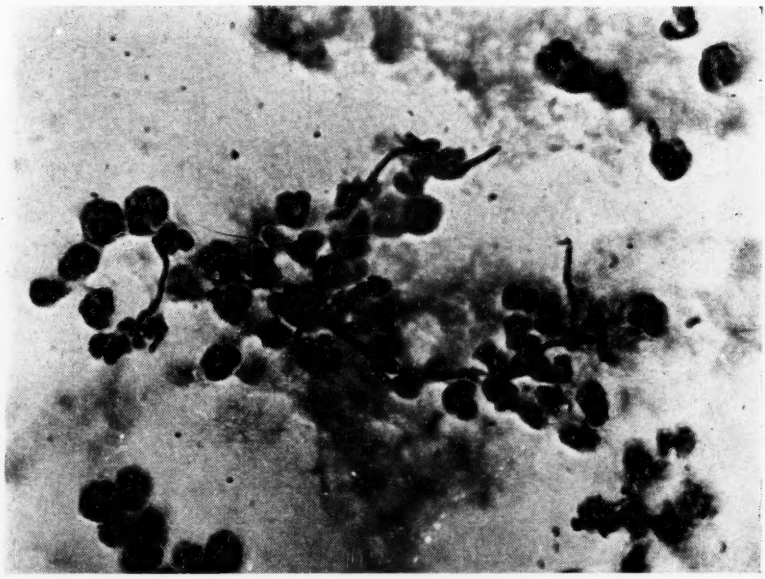

Fig. 1. — Pus from the lung abscess with filamentous bacterium. Gram-staining. Magnification × 1000.

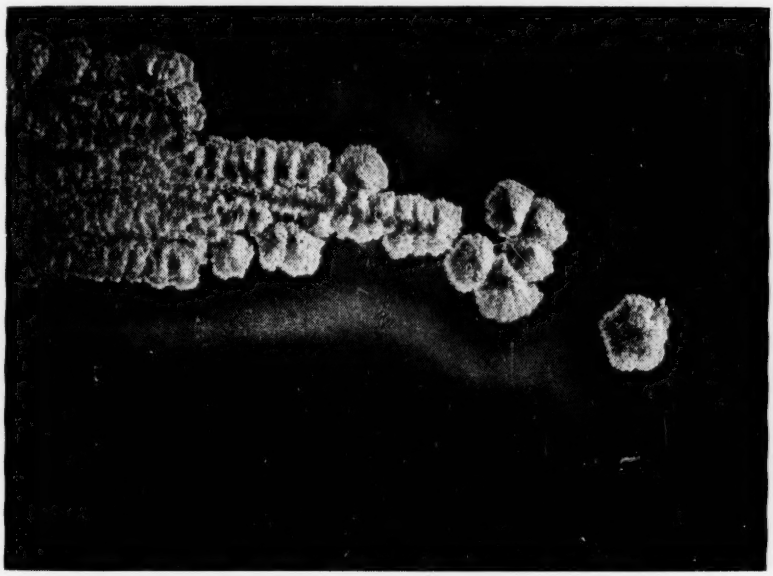

Fig. 2. — R-colonies of the bacterium isolated from the lung abscess on a blood plate. Grown aerobically for 4 days at 37° C. and in a box where carbonic acid was developed. Magnification × 4.

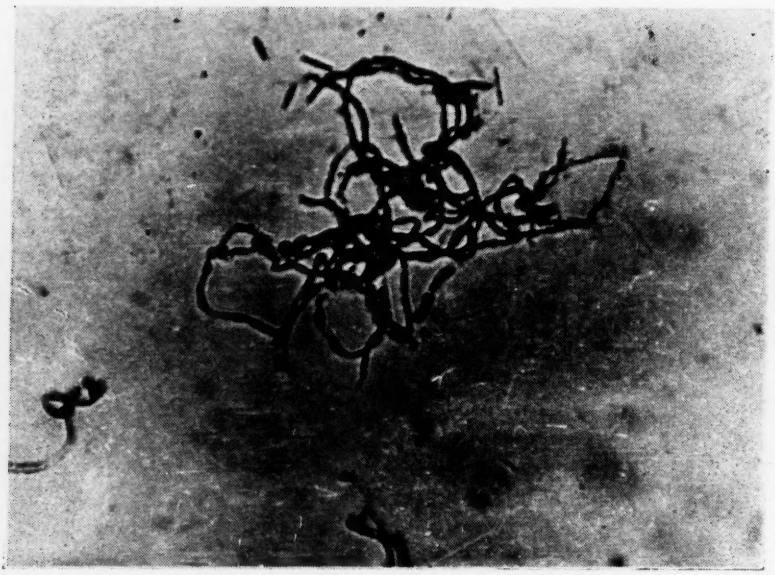

Fig. 3. — Preparation of the R-colony of the bacterium isolated from the lung abscess. Grown aerobically on a blood plate for 4 days. Gram-staining. Magnification × 1000.

upon the medium and the stage of growth. In small S-colonies the bacterium was either rod-shaped or a short filament. In larger R-colonies it had grown into long filaments which occurred in bunches (Fig. 3). Even then the single filaments did not form actual ramifications. Later the filaments crumbled, as it were, into rods of different lengths. In the vigorous phase of growth the heads of the rods and filaments were club-shaped and curled up. In fluid media the bacterium grew only into short filaments.

Following Ørskov's (16) advice, I studied the morphology of the bacterium in different stages of generation, also in fresh colonies and in new cultures of strains which were several months old, but it never ramified in the manner typical of actinomyces. This was also the case when it was cultivated in a living and in a boiled hen's egg, where, according to Wolff and Israel (27), actinomyces ramify best.

The bacterium studied had the following bio-chemical characteristics: It fermented acid from glucose, lactose, saccharose, maltose, mannite, and dextrine. The bacterium did not ferment starch nor

did it develop indole from casein. An acid coagulation was caused in milk in 1—3 days. The NH_3 and H_2S reactions were negative. The bacterium did not dissolve gelatine, nor did it reduce nitrates. The methyl red test was positive and the Voges Proskauer test negative. The optimal growth of the bacterium was at 37° C, but it grew poorly at 45° C and not at all at 18° C. It tolerated one hour's boiling at 55°, but not boiling for 30 minutes at 60° C. The terminal *p*H in a fluid medium was 3.5, which was reached within 7 days. The bacterium remained viable for 3 days in a medium whose acidity was up to 1 per cent (*p*H 2.0) of acetic acid. In acid staining after steaming in carbol fuchsin the bacterium did not tolerate the briefest dissolution in 5 per cent H_2SO_4. The micro-organism was non-sporiferous and immobile.

The pathogenicity of the isolated bacterium was studied in 25 animal tests, which are shown in Table 1. All 17 mice fell ill, but recovered with the exception of 4. The bacterial inoculation caused no local inflammation in the mice apart from slight peritonitis in one, but each of the mice, instead, was found to have developed pulmonary inflammation. Macroscopically a number of pneumonia infiltrations, always non-purulent, were usually seen in both lungs. In 10 tests a bacteriological study was made of the pulmonary infiltrations, and in 3 cases the bacterium inoculated was found to grow as the only micro-organism, as a pure culture, and in 2 culture-negative cases it was seen in the smear. The *E. coli* infections were probably due to intestinal perforation which had occurred at the inoculation. This is indicated by the fact that *E. coli* grew not only in the lung of one dead mouse but also in the peritonitis exudate. The staphylococcus, streptococcus, and the mixed flora, each of which grew once from the lung of mouse, were possibly secondary infections.

Of 3 guinea pigs 2 developed pneumonia, but the bacterium with which they had been inoculated could not be found in their lungs. In contrast to what was noted about the mouse, the bacterium, when inoculated into the guinea-pig subcutaneously, caused a local inflammation. The rabbit also developed pneumonia, but only from a large amount of bacteria injected intravenously. The bacterium in question seemed to have no effect on the rat, at least not in the doses given.

TABLE 1

ANIMAL TESTS MADE ON THE BACTERIUM ISOLATED FROM THE PULMONARY ABSCESS

Test Animals	Inoculation Method and Amount of Bacterial Solution in cc	Effect on the Animals	Examination of the Animals	Bacterial Examination of the Animals
7 mice	Subcutaneously 1.0	All were taken ill after 2—3 days. One died on the 5th day while the rest recovered within a fortnight on an average. No inflammation was noted in any of them at the point of inoculation	All had pneumonic infiltrations in the lungs	In two, the inoculated bacterium grew from the pulmonary infiltration as a pure culture and in one case (the dead mouse) this bacterium was seen in the smear from the infiltration, but did not grow in the cultures. — In one, Staphylococcus aureus grew from the lung and in one a mixed flora where the inoculated bacterium did not grow. No bacterial examination was made in 2 of the cases.
10 mice	Into the abdominal cavity 1.0	All were taken severely ill after 2—3 days. 3 died (a. on the 4th day, b. on the 13th day, c. on the 18th day) and 7 recovered within 3 weeks on an average	All had pneumonic infiltrations in their lungs and one dead animal (c) had an atrophied left lung, adhering tightly to the pleura. Two other dead animals had developed peritonitis besides the pulmonary changes, a. slight and b. purulent	The inoculated bacterium grew as a pure culture from the lung of one dead animal (c). The bacterium was seen in the smear made from the lung and peritonitis exudate of the one dead animal (a), but did not grow in cultures. E. coli grew from the lung and peritonitis exudate of the third dead mouse (b). The lungs of the two which recovered were culture-negative and E. coli grew from the lungs of one. In 4 cases no bacterial examination was made

3 guinea pigs	a. Subcutaneously 1.5	Was taken severely ill and an abscess formed at the point of inoculation	Was examined 2 weeks after the inoculation when the animal was on the road to recovery. The abscess had cicatrized under the skin and there were numerous small pneumonic infiltrations in both lungs	Examination of the pus in the acute stage of the abscess: The inoculated bacterium in the smear, but it did not grow in the cultures. No bacterial growth from the pulmonary infiltrations.
	b. Into the abdominal cavity 2.0	Fell severely ill but began to recover	Was examined 14 days after the inoculation. Nothing noteworthy in the abdominal cavity, but in both lungs pneumonic infiltrations and adherences to the pleura	Streptococcus α — and non-haemolyticus grew from the pulmonary infiltrations
	c. Subcutaneously 0.5 and into the abdominal cavity 0.5	Fell slightly ill and an infiltrate developed at the point of inoculation. Recovered and the infiltrate disappeared	Nothing remarkable could be noted in the killed animal 2 months after the inoculation	No bacteria in the smear from the scar of the subcutaneous infiltrate and no growth in cultures
3 rabbits	a. Intravenously 2.0 and subcutaneously 1.5	No effect was observed	6 weeks after the inoculation an examination of the killed animal revealed nothing pathological	No bacterial examinations
	b. Inolucation directly into the lung 1.5	—»—	—»—	—»—
	c. Intravenously 5.0	Was taken severely ill after 2—3 days, but began to recover towards the end of the second week	Examination of the animal killed 14 days after the inoculation: Clear fluid and fibrin clots in both pleural cavities; no adherences. Numerous pneumonic infiltrations in both lungs, especially in the lower parts	No bacteria grew in cultures from the infiltrations of either lung, nor were any seen in the smear
2 rats	a. Subcutaneously 1.0	No effect was observed	Nothing pathological was noted 2 months after the inoculation in the examination of the killed animal	No bacterial studies
	b. Into the abdominal cavity 1.0	—»—	—»—	—»—

A histologic examination was made of the pulmonary changes occurring in 6 of our test animals.[1] The disease of the following 4 had evidently been caused by the inoculated bacterium alone. The mouse which died on the fourth day had diffuse early pneumonia. In this case the cultures from the lungs were negative, but the bacterium inoculated was visible in the smear. Two mice and one rabbit, which were already recovering and on which the bacteriological examination was negative, had slight focal pneumonia, probably in the late stage. Two mice had pneumonia which was morphologically different from the one manifested in the foregoing instances. *E. coli* was cultivated from the lungs in the two cases mentioned last.

The bacterium isolated from our patient's lung abscess had a resistance to penicillin of 0.15 U/cc, while that of the control, Oxford strain *Staphylococcus aureus* H, was 0.075 U/cc. Its resistance to streptomycin was 1.5 microg/cc, while that of our own laboratory strain *E. coli* 3453 was 3.0 microg/cc. The resistance of the filament bacterium to sulfathiazole and to Elkosin was 2.5 mg per 100 cc, the resistance of *Staphyloc. aureus* H being the same.

While the patient was being treated with antibiotics, tests were made on her serum to discover its power of inhibiting bacterial growth. It appeared from several tests that the penicillin concentration of the serum to affect the filamentous bacterium had to be 3—4 times larger than was implied by the resistance value previously determined (0.15 U/cc), irrespective of the fact that the limit of inhibition to the growth of the control bacteria agreed with their resistance values. Thus as much as 125,000 U × 8 was needed to obtain a sufficient penicillin concentration in the serum. Because of this the patient was treated with large doses of penicillin. The serum tests further showed that penicillin inhibited the growth of the isolated bacterium equally well when alone as in conjunction with Elkosin. The streptomycin present in the patient's serum inhibited the growth of the bacterium in a concentration corresponding to the resistance value.

The possible antibiotic effects of the bacterium on *Staphyloc. aureus* and *E. coli* were studied. The results obtained were negative.

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[1] E. K. Ahvenainen, M.D. examined the histologic preparations, for which I wish to express my thanks.

Agglutination tests, using as antigen the bacterium isolated from the lung abscess, were made on the patient's serum 2½ months after the treatment had ended. Eight normal sera produced agglutination almost equally advanced as the patient's serum, so that no specific agglutination could be proved, at least not so long after the recovery of the patient from her disease.

DISCUSSION

A closer study of the bacterium isolated from the lung abscess showed that contrary to what was first supposed, the patient did not suffer from pulmonary actinomycosis. The bacterium did not form true branches, in the manner characteristic of actinomyces, either in the lung or in the cultures. Actinomycosis was thus ruled out, as it was in the histologic examination of the necrotic lung tissue.

For identification I also took into account bacteroides (*Bact. melaninogenicum, Bact. fragilis, Bact. necrophorus*) and fusobacteria, which have frequently been met in pulmonary abscess (4, 23, 24), but these are Gram-negative and strictly anaerobic. The fusobacterium, moreover, is a straight, tapering filament, which always develops indole and hydrogen sulfide (1, 2, 6). The morphology of our bacterium might perhaps have conformed with leptotrichia, but unlike them it dissolved mannite, did not ferment starch and did not grow even on the »Difco» medium in the serpent-like or medusa-like colonies typical of leptotrichia (22).

We finally inferred that the morphologic, cultural, and biochemical properties of the bacterium isolated from the abscess corresponded best to those of lactobacilli (2, 19, 21, 26, *et al.*). According to bacteriological literature it seems best to classify lactobacilli obtained from the human mouth and intestines, to which group our lung abscess bacillum apparently belongs, according to their oxygen requirement (11, 19, 25, 26). Our bacterium, accordingly, belongs to the microaerophil *Lactobac. acidophilus* species and is not a species of the anaerobic *Lactobac. bifidus* type II. The special tendency of the bacterium to form long filaments does not contradict this identification, for a species of *Lactobac. acidophilus* with similar properties has been met with and has been called »filamentous» or »type X» (7, 12, 17, 18).

It was exceptional that there should have been a monoinfection in the lung abscess. From numerous pus samples we disclosed the lactobacillus alone both in smears and in cultures. Only when the patient was recovering, when the bacterium was gradually disappearing, did other bacteria appear. A pulmonary abscess traceable to aspiration has usually been found to contain a mixed flora, almost invariably including anaerobic bacteria (4, 15, 23, 24). The less frequent haematogenic lung abscess, on the other hand, is originally traceable to one single bacterium (*Staphyloc. aureus*, *Streptoc. β-haemolyticus*). May it not be possible that other bacteria, besides, e.g. an anaerobic streptococcus or *Bact. melaninogenicum* originally existing in the abscess went unnoticed or disappeared before the first examination of the abscess content, while our patient was being treated with penicillin? Against this stands the fact that the treatment was of short duration only, and that it was started with small doses of penicillin, and secondly, that in the sputum and the punctates there was no foul smell such as is caused by the bacteria mentioned, characteristic of anaerobic gangrene. Our bacterial strain had no antibiotic effect, but it is possible that its great capacity to develop acid was the reason for the inviability of other bacteria in the abscess cavity.

The fatal pulmonary tuberculosis which our patient developed later was evidently a new disease. One can understand how her diabetes and the condition following the lung abscess should have made her prone to tuberculosis.

In contrast to what is generally supposed the lactobacillus, apparently the cause of our patient's lung abscess, was pathogenic for man. There has been some discussion about the part played by *Lactobac. acidophilus* in dental caries, but I have only met in the literature one actual instance of a disease ascribable to this bacillus. This was a case of endocarditis described by Marschall (14), where the disease was caused by *Bacillus Döderlein*, regarded by many bacteriologists as a species of *Lactobac. acidophilus* (5, 19, 26).

In their investigations on the pathogenicity of *Lactobac. acidophilus*, Rosebury *et al.* (20), Canby and Bernier (3) and King and Rettger (11) noted that in the test animals it gave rise to subcutaneous abscesses from which the inoculated bacterium no longer grew in cultures. Further, Jay *et al.* (10) noted that the R-strains but not the S-strains of this bacterium caused similar abscess

both in the rabbit and in man. The results are in agreement with those we obtained with our own lactobacillus strain in experiments on guinea pigs.

It was noteworthy that our lactobacillus caused pulmonary inflammation in the mouse, the guinea pig, and the rabbit. Not once did the pneumonic infiltrations of the animals form abscesses, which may be due to the fact that the bacterium lost some of its virulence in the laboratory. In Howitt and Van Meter's (8) interesting experiments the lactobacilli obtained from the human mouth and intestines when injected into the rabbit's vein sometimes settled either in the liver, the kidneys, or the lungs. Some of their strains had an especial affinity to the joints of the rabbit, in which muco-purulent arthritis developed. Thus pathogenic lactobacilli actually seem to exist and manifest an affinity to various organ.

Lactobac. acidophilus type X appears to be a fairly common bacterium in the human alimentary canal, and it probably occurs to some extent in the respiratory passages also, without giving rise to any diseases. My studies on the bacterial flora of patients with gastroduodenal ulcer also seem to point to this (13). I isolated 7 lactobacillus strains of this type from the stomach or from the peritonitis exudate of 4 out of 48 patients. Judging from the case now described the virulence of this bacterium may be enhanced under certain conditions and it may cause severe diseases, pulmonary inflammations in particular. Is it possible that the high sugar content of our patient's tissues may have increased the virulence of the bacterium? We know from our studies of the resistance of the bacterium and from experience gained in the treatment of the patient that this pathogenic lactobacillus is sensitive to penicillin, streptomycin, and sulfonamides.

SUMMARY AND CONCLUSIONS

The author describes a case with pulmonary abscess in which the sole micro-organism was a Gram-positive bacterium, rod-shaped and the shape of long non-branching filaments. It was a microaerophil bacterium which grew first in small S-colonies in plate cultures, but in a few days changed into α-haemolytic R-colonies 1—3 mm in diameter. Its morphologic, cultural, and bio-chemical properties justified its inclusion among lactobacilli.

It was identified as *Lactobacillus acidophilus* type X. The bacterium was pathogenic in animal experiments and showed an especial affinity to pulmonary tissue. The mouse and the guinea pig contracted pneumonia both from subcutaneous and intra-abdominal inoculation. The rabbit also developed pneumonia, but only from a large dose into the vein.

The author concludes that there is a species of lactobacillus, *Lactobac. acidophilus* type X, which occurs in the human mouth, intestines, and respiratory passages, and may become pathogenic under certain conditions. It manifests a particular affinity to lung tissue and may even cause pneumonia followed by an abscess. This bacterium is sensitive to penicillin, streptomycin, and sulfonamides.

REFERENCES

1. Bøe, J.: Skrifter utg. av det Norske Videnskaps-Akademi i Oslo. I Mat. Naturv. Klasse 1941:No.9.
2. Breed, R. S., Murray, E. G. D., and Hitchens, A. P.: Bergey's Manual of Determinative Bacteriology, 6th Ed., Williams & Wilkins Co., Baltimore, 1948.
3. Canby, C. P., and Bernier, J. L.: J. Am. Dent. A. 1942:*29:*606.
4. Cohen, J.: Arch. Surg. 1932:*24:*171.
5. Cruickshank, J., and Cruickshank, R.: A System of Bacteriology in Relation to Medicine 1931:*8:*334.
6. Dubos, R. J.: Bacterial and Mycotic Infections of Man. J. B. Lippincott Co. Publishers, Philadelphia, London, Montreal, 1948.
7. Howitt, Beatrice: J. Infect. Dis. 1930:*46:*351.
8. Howitt, Beatrice, and Van Meter, Martha: J. Infect. Dis. 1930: *46:*368.
9. Jay, P., Crowley, Mary, and Bunting, R. W.: J. Am. Dent. A. 1932:*19:*265.
10. Jay, P., Crowley, Mary, Hadley, F. P., and Bunting, R. W.: J. Am. Dent. A. 1933:*20:*2130.
11. King, J. W., and Rettger, L. F.: J. Bact. 1942:*44:*301.
12. Kopeloff: Lactobacillus acidophilus. Williams & Wilkins Co. 1926. — Reported by Howitt (1930).
13. Korttila, K.: Acta Chir. Scand. 1951:Suppl.163.
14. Marschall, F.: Zbl. f. Bakt. 1938:*141:*153.
15. Meleney, F. L.: Clinical Aspects and Treatment of Surgical Infections. W. B. Saunders Co., Philadelphia, London 1949.
16. Ørskov, J.: Investigations into the Morphology of the Ray Fungi. Levin & Munksgaard Publishers, Copenhagen 1923.
17. Rettger, L. F., and Horton, G. D.: Zbl. f. Bakt. 1914:*73:*362.

18. Roos, C.: J. Lab. & Clin. Med. 1926/27:*12:*1053.
19. Rosebury, T.: Arch. Path. 1944:*38:*413.
20. Rosebury, T., Foley, Genevieve, and Greenberg, S.: J. Dent. Research. 1934:*14:*231.
21. Thjøtta, Th.: Lærebok i Bakteriologi. Bind II. Spesiell Bakteriologi. Fabritius & Sønners Forlag, Oslo, 1945.
22. Thjøtta, Th., Hartmann, O., and Bøe, J.: Skrifter utg. av det Norske Videnskaps-Akademi i Oslo. I Mat. Naturv. Klasse 1939: No.5.
23. Valle, A. R.: Surg., Gynec. & Obstr. 1945:*81:*278.
24. Varney, P. L.: Arch. Surg. 1929:*19:*1602.
25. Weiss, J. L., and Rettger, L. F.: J. Infect. Dis. 1938:*62:*115.
26. Wilson, G. S., and Miles, A. A.: Topley and Wilson's Principles of Bacteriology and Immunity. Vol. I. 3th Ed. Edward Arnold & Co., London, 1947.
27. Wolff, M., and Israel, J.: Virchows Arch. 1891:*126:*1.

FROM THE WIHURI RESEARCH INSTITUTE, HELSINKI

EFFECT OF CORTISONE ON FAT ABSORPTION AS EVIDENCED BY CHYLOMICROGRAPHIC STUDIES

by

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It has long been known that the adrenal cortex in some way affects the metabolism of fat. Adrenalectomy prevents the development of a fatty liver in experimental animals both in experimental phosphorus poisoning and after the removal of the pancreas (36). A fatty liver can be produced, if the adrenalectomized animals of the above experiments are given adrenocortical hormones.

Information on the effects of cortisone and ACTH on the human fat metabolism is still rather meagre. It has been claimed that cortisone raises the serum cholesterol level (1, 28), or that it has no effect on it (32), or that it lowers it (7). Adlersberg *et al.* also mention that cortisone raises the blood phospholipid level and has an opposite effect on the neutral fat. According to them, ACTH has a similar though weaker action as cortisone.

It is only natural that the actions of cortisone and ACTH on fat metabolism have been more extensively investigated in experimental animals. It has been demonstrated that cortisone produces a fatty liver in rabbits (25), but not in mice (17). Best and Campell (6) and Raab (24) had already shown that pituitary extracts cause the development of a fatty liver in experimental animals, which depends on a transfer of stored fat into the liver (4). It has been shown that ACTH produces a fatty liver in the rat (3), in the mouse (17) and in the rabbit (8). Levin and Farber (17) have demonstrated that ACTH is not able to produce a fatty liver in adrenalectomized mice, but if cortisone is administered simultaneously to such a mouse, a fatty liver will develop. According to them, the ability of ACTH to produce a fatty liver is not mediated through

the adrenal cortex, but in order to induce a fatty liver, either exogen or endogen cortisone is needed, in addition to the ACTH.

It is also known that the adrenal cortex affects the intestinal absorption of fat. Laszt and Verzar (16) and Montini and Pontremoli (19) have shown that fat remains unabsorbed in the intestine after adrenalectomy. If such an adrenalectomized rat is given adrenocortical hormones, fat will again be absorbed. Frazer (11) mentions that adrenalectomy reduces the intestinal absorption of triglycerides, but that it has no effect on the absorption of fatty acids.

Earlier investigations seem thus to indicate that the adrenal cortex has a significant rôle in the metabolism of fat.

A convenient method for the study of the absorption of fat is the observation of chylomicronaemia in the blood after the administration of fat. This method is probably been first used by Neuman (21) and after him by several others (5, 12, 13, 23, 26, 33) in investigations on fat absorption or fat metabolism. Since the action of cortisone on chylomicronaemia after the administration of fat has evidently not been studied, the present problem was considered interesting and worth examining.

METHOD, MATERIAL, AND RESULTS

The chylomicrons were examined in a sample of blood taken from the tip of the rat's tail before the administration of olive oil and after it hourly, until the chylomicrons had again disappeared from the blood. A small drop of blood was taken on a cover glass, which was then turned over on a slide, when the blood spread over the whole surface of the cover glass. In order to obtain a thin smear, the cover glass was lightly pressed with clean rubber. The edges of the cover glass were treated with paraffin wax. Both the cover glasses and the slides had been cleaned carefully and they had no scratches. The samples were examined immediately after taking, as it has been observed that the number of chylomicrons is reduced when the preparation is left standing (29). The chylomicrons were counted in a dark field by using 2 280 $\times$ magnification. A ruling was attached to the ocular of the microscope, with the used magnification the size of each square was 8×8 μ. The number of chylomicrons is expressed in this work as per one square. In each preparation, 20 squares were counted, and the mean result is

given. The chylomicrons were always counted in similar parts of the preparations, in which the erythrocytes were immobile and separated from each other by long distances of serum. With this method we have obtained, according to our judgement, the most reliable results.

In the present work we have been defined as chylomicrons all those smaller and larger particles which are visible in the dark field with oil immersion and 2 280 × magnification, whether bright or dark, if they are in Brownian movement. Similarly Elkes, Frazer and Stewart (9) have counted as chylomicrons all particles of various sizes appearing in connection with the administration of fat.

Since the counting of chylomicrons in the dark field requires experience, all the chylomicron counts were made by one of us (R.H.), who has made such counts during a longer time in an other connection. Considerable errors may occur, when the number of the chylomicrons per field becomes high. However, it is probable that a dilution of the serum gives too high chylomicron counts (10), and therefore we have not diluted our samples. An attempt was made to avoid the above source of error by selecting, through trial the amount of olive oil given as so small (0.07 cc per 100 g of body weight) that too high chylomicron counts were avoided. When judging the differences of the chylomicron counts, small differences must not be attached much importance. Differences up to 30 per cent may be accidental.

Thirty male albino rats of the same age and breed, weighing 180 to 210 g, were used in the experiments. During the whole work the rats were kept on a diet poor in fat. They were fasted for 18 hours before the administration of fat. Before beginning the cortisone experiments, a control administration of fat was made on each group of 6 rats, and the later results were compared with these control values. The dose of fat was in all experiments 0.07 cc of olive oil per 100 g of body weight, and it was given with a tuberculin syringe through a rubber catheter directly into the stomach under aether anaesthesia. In two experiments the olive oil was accidentally given into the lungs, and these cases have been excluded from the material.

Cortisone (Cortone »Merck») was injected into the rats subcutaneously in the following manner: (1) 2 groups were given 5 mg

as a single dose 4 hrs before the administration of fat; (2) 1 group 2.5 mg pro die; (3) 2 groups 5 mg pro die. After a dose smaller than 2.5 mg no distinct changes were observed in comparison to the control curves. The administration of fat was made on the 4th, 7th and 11th day after starting the 5 mg pro die injections of cortisone, and on the 7th day in the group receiving 2.5 mg pro die.

The liver of the rats was preserved for a histological examination. The slides were stained with scarlet red in frozen sections.

It was observed that microchylomicrons occurred both during the rise and during the fall of the chylomicronaemia, whereas the maximum of the chylomicron curve was characterized by the presence of almost only macrochylomicrons.

The chylomicron curves of all the rats given cortisone are not presented, because of essential similarity. The figures (Figs. 1—3) show, as an example chylomicron curves, obtained on one of the groups of 6 rats which were given 5 mg of cortisone pro die. Fig. 1 shows the chylomicronaemia in an experiment which was started 4 hrs after the administration of cortisone, and the following figures (Fig. 2 and Fig. 3) that after the animals had received cortisone during 4 and 11 days. Fig. 4 shows the chylomicron curves of 6 rats which had received 2.5 mg of cortisone pro die during 7 days.

No chylomicrons were to be seen in the blood of fasting rats in the controls or after the administration of cortisone. After the administration of fat there obtained a great difference in the chylomicronaemia between the cortisone rats and the controls. The chylomicron curves of the cortisone animals were much higher and more protracted. The longest and highest curves were observed in the rats which had received the larger dose of cortisone. The length and the height of the curve seemed to depend also to some extent on the duration of the cortisone treatment. It may be pointed out that a distinct rise of the chylomicron curve occurred already in the experiment made 4 hrs after the administration of 5 mg of cortisone.

DISCUSSION

It is rather difficult to explain why the cortisone group showed a higher and more protracted chylomicron curve. This depends on the fact that the mechanism of the absorption of fat is still an

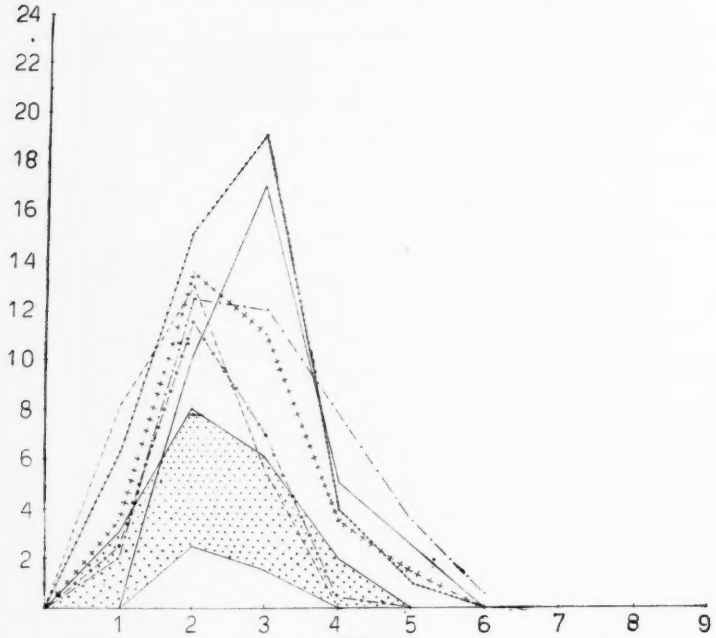

Fig. 1.

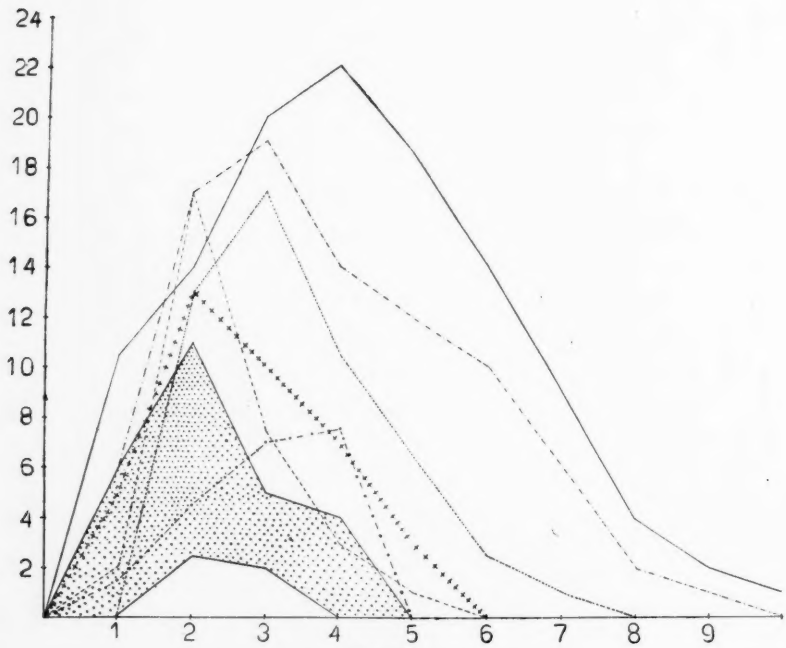

Fig. 2.

Figs. 1 to 4. — Chylomicrograms. The number of the chylomicrons on the ordinate, time in hours on the abscissa. The spotted area shows the range of the chylomicrograms of the untreated rats. Each of the curves shows the

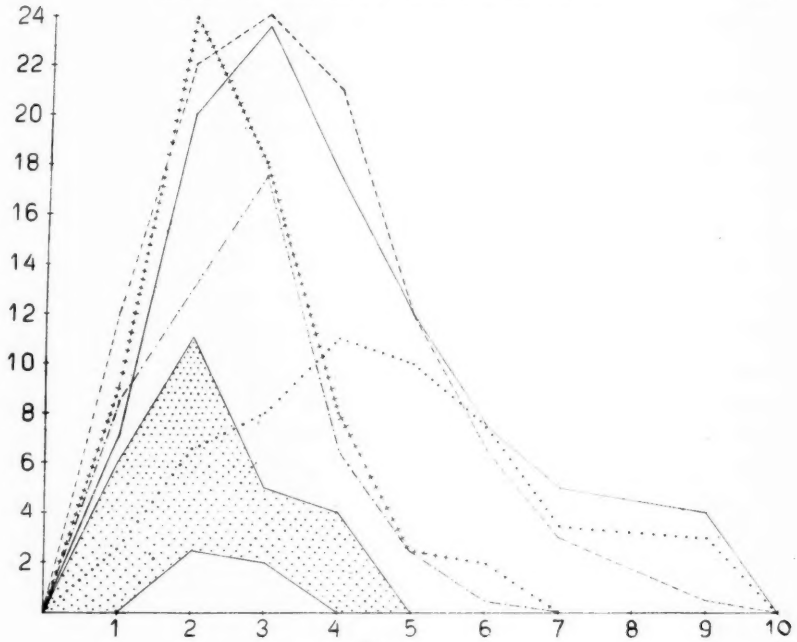

Fig. 3.

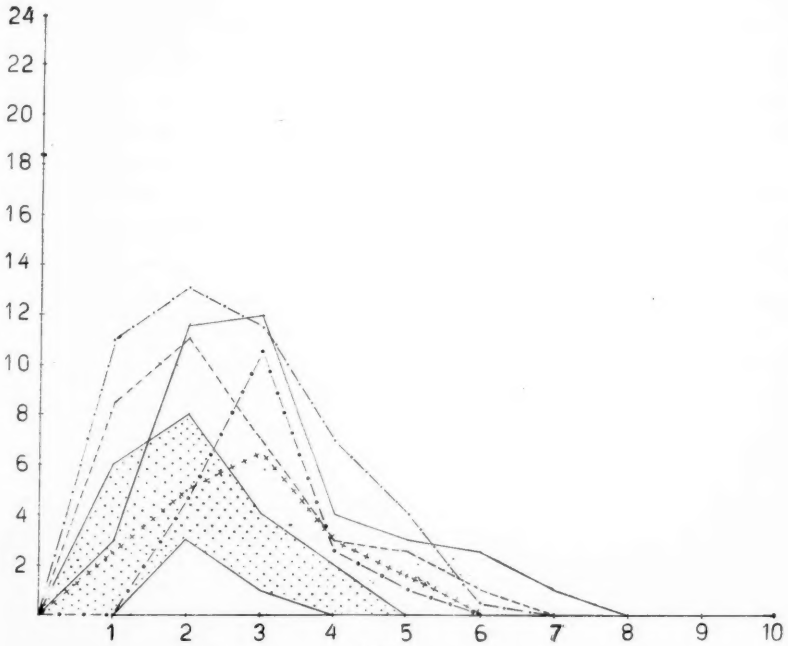

Fig. 4.

chylomicrogram of a single rat after the subcutaneous administration of cortisone. Fig. 1: 5 mg of cortisone as a single dose. Fig. 2: 5 mg pro die during 4 days. Fig. 3: 5 mg pro die during 11 days. Fig. 4: 2.5 mg pro die during 7 days.

unsolved problem and that the actions of cortisone are in many respects poorly understood. Information on the physiological significance of the chylomicrons in the metabolism is also meagre. Moreover, various views are still presented as to the chemical composition of the chylomicrons. Frazer(11) and Swank and Wilmot (30) claim that they contain mainly neutral fat, according to Moreton (20) they are in the main cholesterol, and according to Marder *et al.* (18) triglycerides.

The absorption of fat is at the present explained by applying either of two theories, which both have their supporters. The lipolytic theory, which is based on Pflüger's classical presentation, explains the absorption as occurring so that the fat is first emulsified and then hydrolyzed into fatty acids and glyzerol, and then absorbed. The fatty acids attached to the bile pass into the epithelial cells of the intestinal villi where they undergo phosphorylation (27), and they are the resynthetized into neutral fat, which is transported through the lymph vessels into the liver. Frazer's partition theory again maintains that fat is emulsified in the intestine and hydrolyzed as well partially as completely. The fatty acids are absorbed and pass through the portal circulation into the liver, whereas the emulsified glyzerides, with particle diameters of less than 0.5 μ, are absorbed through the intestinal mucosa directly into the lymphatic system.

Several hypotheses can be presented to the explanation of the high postprandial chylomicron curves of the cortisone rats. An increased absorption of fat seems a probable explanation, since, as mentioned above, adrenlectomy is known to cause disturbances in the absorption of fat. The findings that cortisone quite soon effects a change in the curve agrees well with this view. If one applies the lipolytic theory, it is possible to explain the increased absorption, since the phosphorylation of fatty acids in the intestinal mucosa is an important factor in their transportation, and this again is known to be regulated the adrenal cortex (1, 29). However, if one attempts to explain the changes in the chylomicronaemia with the aid of Frazer's theory, the high curves could be ascribed to a changed partition of the fat between the portal circulation and the lymphatic systems and thus to a high chylomicron curve.

Instead of an increase in the absorption of fat, the high chylo-

micron curves may be explained also in other ways. It can be suggested that cortisone prevents the storage of fat or that it reduces the use of fat already absorbed. The former possibility does not seem likely, as it has been previously shown that adrenalectomy prevents the storage of fat (34). This view is made untenable also by our observation that increased amounts of fat were to be seen in the liver of the cortisone treated rats. It might also be suggested that cortisone first fills the stores of fat, and thus an additional storage of fat would become hampered. It is interesting to notice, that cortisone also produces an intense glycogen deposition in the liver (22, 31, 35). According to Kerppola (15), 10 to 20 mg of cortisone pro die increases the liver glycogen of rabbits by 20 to 30 times. An increase of the dose of cortisone over this does not produce any higher effect on the liver glycogen. A reduction in the use of fat is again made unlikely by the observation that cortisone leads to increased ketosis, which is explained as being caused just by an increased utilization of fat (25).

It has been recently reported that heparin *in vivo* causes a clearing of lipaemic sera and decrease in the number of chylomicrons (2, 14, 29). It is not known whether cortisone perhaps would counteract this action of heparin.

Theoretically, it can also be suggested that cortisone may cause an increase in the size of chylomicrons and then perhaps particles which had been previously invisible would become visible, thus raising the chylomicron curve.

SUMMARY

Chylomicronaemia and the action of cortisone on it were studied in 30 rats. Cortisone, administered subcutaneously in a dose of 2.5 or 5.0 mg per rat during 1 to 11 days, caused a considerable increase in chylomicronaemia after the administration of fat. The heigh and the length of the chylomicron curve seemed to increase with rising dose and increasing duration of the administration of cortisone. The chylomicron curve was higher and more protracted than in controls already after a single dose of 5 mg of cortisone.

BIBLIOGRAPHY

1. Adlersberg, D., Schaefer, L. E., and Dritch, R.: Proc. Soc. Exper. Biol. & Med. 1950:*74:*877.
2. Anderson, N. G., and Fawcett, B.: Proc. Soc. Exper. Biol. & Med. 1950:*74:*768.
3. Baker, B. L., Ingle, D. J., Li, C. H., and Evans, H. M.: Am. J. Anat. 1948:*82:*75.
4. Barret, H. M., Best, C. H., and Ridout, J. H.: J. Physiol. 1938: *93:*367.
5. Becker, G. H., Meyer, J., and Necheles, H.: Science 1948:*110:*529.
6. Best, C. H., and Campbell, J.: J. Physiol. 1936:*86:*190.
7. Conn, J. W.: quoted by Rich, A. R. et al.: Bull. Johns Hopkins Hosp. 1951:*88:*101.
8. Diczfalysy, E., and Westman, A.: Lancet 1950:*II:*541.
9. Elkes, J. J., Frazer, A. C., and Stewart, H. C.: J. Physiol. 1939: *95:*68.
10. Frank, H., and Ingelfinger, F. J.: J. Lab. & Clin. Med. 1950:*35:*815.
11. Frazer, A. C.: Physiol. Rew. 1946:*26:*103.
12. Frazer, A. C., and Stewart, H. C.: J. Physiol. 1937:*90:*18.
13. Gage, S. H., and Fish, P. A.: Am. J. Anat. 1924:*34:*1.
14. Hahn, P. F.: Science 1943:*98:*19.
15. Kerppola, W.: To be published in Endocrinology.
16. Laszt, L., and Verzar, F.: Biochem. Ztschr. 1936:*288:*351.
17. Levin, L., and Farber, R.: Proc. Soc. Exper. Biol. & Med. 1950: *74:*758.
18. Marder, L., Becker, G. H., Maizel, B., and Necheles, H.: Gastroenterology 1952:*20:*43.
19. Montini, T., and Pontremoli, S.: Boll. Soc. ital. biol. sper. 1951: *27:*704.
20. Moreton, J. R.: Science 1948:*107:*371.
21. Neuman, A.: Wien. Klin. Wchnschr. 1907:851.
22. Olson, R. E., Thayer, S. A., and Kopp, L. J.: Endocrinology 1944: *35:*464.
23. Parviainen, S., and Ermala, P.: Ann. Chir. et Gynaec. Fenniae 1951:*40:*245.
24. Raab, W.: Klin. Wchnschr. 1934:*13:*281.
25. Rich, A. R., Cochran, T. H., and McGoon, D.: Bull. Johns Hopkins Hosp. 1951:*88:*101.
26. Setälä, K.: Radiology 1948:*50:*803.
27. Sinclair, R. G.: Physiol. Rew. 1934:*13:*351.
28. Sprague, R. G., et. al.: quoted by Rich, A. R. et al.: Bull. Johns Hopkins Hosp.: 1951: *88:* 101.
29. Swank, R. L.: Am. J. Physiol. 1951:*164:*798.
30. Swank, R. L., and Wilmot, V.: Am. J. Physiol. 1951:*167:*403.

31. THEILUM, G., ENBÆK, H. C., and SIMONSEN, M.: Acta Endocrinol. 1950:*5:*151.
32. THORN, G. W.: quoted by RICH, A. R. et al.: Bull. Johns Hopkins Hosp. 1951:*88:*101.
33. TIDWELL, H.: J. Biol. Chem. 1950:*182:*405.
34. TUERKISCHER, E., and WERTHEIMER, E.: J. Physiol. 1942:*100:*385.
35. VENNING, E. H., KAZMIN, V. E., and BELL, I. C.: Endocrinology 1946: *38:*79.
36. VERZAR, F., and LASZT, L.: Biochem. Ztschr. 1936:*288:*356.

THE FISH TAPEWORM

OCCURRENCE IN CHILDREN IN THE FINNISH LAKE AREA

by

ESKO TÄHTI

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The area of Hankasalmi parish in Central Finland amounts to 602 sq. km (i.e. 226.43 sq. mi.). A considerable part of it consists of lakes. The number of inhabitants is 9,868.

Fig. 1.

Acting as a district medical officer I microscoped during last winter the stools of 1,226 elementary school children. The ages of the examined pupils varied between 7—15 years. As a rule, only one stool specimen of each pupil was examined.

194 pupils or 15.8 per cent in all were found to be infested with the fish tapeworm (*Diphyllobothrium latum*). Considerable variations as to the frequence were observed in the various school districts. In order to study what possible influence the watersystems had on the incidence of the fish tapeworm disease, I counted the percentage of the lake area in each school district. The following graph shows the correlation between the occurrence of the fish tapeworm and the percentage of the water covered areas.

There seems to be a certain increase in the incidence of the fish tapeworm disease as the lake-covered area increases, although the curve does not rise regularly.

Further limnological investigations, as well as a geographical analysis of the lake systems, will perhaps throw some additional light on the curve.

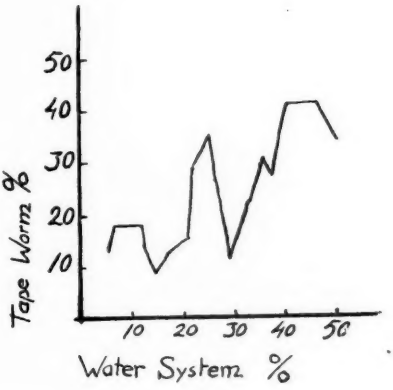

Fig. 2.

FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF HELSINKI

PREGNANCY AND ANAPHYLAXIS IN GUINEA-PIGS

by

DANIEL ECKERT and M. K. PAASONEN

(Received for publication September 8, 1952)

Few investigators have paid attention to the effect pregnancy has on anaphylaxis. Lumiere and Couturier (8) noticed 1921 that the guinea-pigs remaining alive and showing little or no sign of shock after reinjection when sensitised with horse serum, were all pregnant. To study the effect of pregnancy on anaphylactic shock more closely, Lumiere and Couturier sensitised male and pregnant female guinea-pigs with normal horse serum. Although all the males died of anaphylactic shock after an intracardial re-injection with 0.6 ml of the same serum, the pregnant females had symptoms of mild shock only. When the pregnancy ended the animals reaction to a reinjection was again an anaphylactic shock. Sensitized males injected with plasma of pregnant guinea-pigs and an antigen were not protected from anaphylactic shock. The writers drew the conclusion that the »immunity» they noted during pregnancy was not the result of humoral factors but of the weakening of the reflexes and of what was happening in the nervous system. Lumiere and Couturier, however, give no details of their experiments in their publication. Ratnoff (11) sensitized rats with normal horse serum between 24 and 36 hours after mating and found no difference between the reaction of pregnant and control animals to reinjection. Pylkkö and Vartiainen (9) showed that pregnant guinea-pigs were much more resistant to histamine than nonpregnant females, or males. They noticed

also that four out of five male guinea pigs sensitised with normal horse serum (0.5 ml) suffered shock on being reinjected intracardially 16 days after sensitization, while none of the five pregnant females did so. According to Cionini (2, 3) guinea-pigs cannot be passively sensitized towards the end of pregnancy with either homologous or heterologous anti-serum. This property, however, they lose within a short time after the birth of their young. Doerr (4) connects this phenomenon with observations made by Kellaway and Cowell (6) on the suppression of anaphylactic symptoms. In fact, normal guinea pig serum injected into a sensitized animal caused the anaphylactic symptoms to disappear for about one day. The urine of pregnant women and an extracts prepared from it administered before the re-injection also definitely moderated the anaphylactic symptoms of sensitized guinea-pigs (12, 13).

This resistance of guinea-pigs to anaphylactic shock during pregnancy seems natural and necessary since placenta albumin for instance, whose antigen character has often been demonstrated, then appears in the blood circulation (7). On the other hand, the suggestion that this resistance is due to a higher histamine tolerance arising from an increased capacity for histaminolysis during pregnancy (1, 15), although such a resistance has been proved (9), seems less feasible for the following two reasons: Histamine does not play a dominant part in anaphylaxis, among the reasons for this being that rutin prevents anaphylactic shock but not histamine shock in sensitized guinea-pigs (5, 10). Secondly, to reserve a protective mechanism for the last stage, i.e. to forestall the consequences of the antigen-antibody reaction, does not appear to be the best way of fulfilling the purpose. It would have been more reasonable to have used the protection at an earlier stage. Here it would be a question of preventing the formation of sessile antibodies and the antigen-antibody reaction.

OWN INVESTIGATIONS

In order to find out whether resistance to anaphylactic shock developed in pregnant guinea-pigs, and if so for what reasons, we sensitised subcutaneously some guinea-pigs with 0.5 ml normal horse serum. To prevent the injected serum from running out of the cut made by the needle we used the 8 cm long injection needle

TABLE 1

THE EFFECT OF PREGNANCY ON THE SENSITIZING EFFECT OF ANTIGEN. GUINEA-PIGS WERE SENSITIZED WITH 0.5 ML NORMAL HORSE SERUM AND REINJECTED INTRACARDIALLY WITH THE SAME AMOUNT OF THE SAME SERUM

Sensitized	Reinjected	Number of Animals	Results of Reinjection					
			No Shock		Non-Fatal Shock		Fatal Shock	
			No.	%	No.	%	No.	%
Non-pregnant	Non-preg.	8	0	0	1	12.5	7	87.5
Non-pregnant	Pregnant	15	0	0	4	26.6	11	73.4
Pregnant	Pregnant	33	13	39.3	11	33.3	9	27.4
Pregnant	After parturition	30	12	40.0	11	36.6	7	23.4

recommended by Thomsen (14) which was pushed for almost its whole length under the skin of the abdomen before the syringe was emptied. The animals were given intracardially another injection of the same amount of the same serum at the earliest 16 days and at the latest 70 days after the sensitization. Both pregnant and normal female guinea-pigs were sensitized in this way. Most of the guinea-pigs sensitized when non-pregnant were put with the males for a period of 10 days from 6 to 10 days after the sensitization. All but two of these were reinjected with antigen towards the end of their pregnancy. Some of the guinea-pigs were reinjected with antigen only 2 to 24 days after parturition. The experiment was performed between December 1950 and July 1951.

We divided the guinea-pigs into four groups according to their condition at the time of sensitization and reinjection:

1) those sensitized and reinjected when non-pregnant
2) those sensitized when non-pregnant but reinjected when pregnant
3) those sensitized and reinjected when pregnant
4) those sensitized when pregnant and not reinjected until after the partus.

The reaction of the different groups to reinjection is given in Table 1. in which we have also entered and classified the results of reinjection. From this Table it appears that reinjection

TABLE 2

QUANTITATIVE EXPERIMENTS ON THE EFFECT OF PREGNANCY ON ANAPHYLAXIS GUINEA-PIGS WERE SENSITIZED WHEN PREGNANT WITH 0.05 ML NORMAL HORSE SERUM DILUTED TO 1 ML WITH PHYS. NACL SOLUTION DEC. 16, 1951. REINJECTED INTRACARDIALLY WITH THE QUANTITY OF HORSE SERUM GIVEN IN THE TABLE, DILUTED TO 1 ML

No.	Partus	Reinjection			Anaphylactic Reaction
		Date	Incubation in Days	Antigen in ml	
1	Dec. 22, 1951	Jan. 1 1952	16	0.10	+
2	»	»	»	0.15	0
3	Dec. 17, 1951	»	»	0.30	+
4	Dec. 23, 1951	»	»	0.50	++
5	Dec. 17, 1951	»	»	0.50	++
6	»	»	»	0.60	+++
7	Dec. 19, 1951	»	»	0.75	+++
8	Jan. 8, 1952	Jan. 15 1952	30	0.30	+
9	Dec. 30, 1951	»	»	0.40	++
10	Jan. 8, 1952	»	»	0.50	++
11	»	»	»	0.70	+++

0 = No visible shock symptoms
+ = Masseter convulsions or quite mild bronchospasms
++ = Severe bronchospasms and protracted shock
+++ = Shock leading to death

of the first two groups had usually fatal consequences whether or not the guinea-pigs were pregnant at the time of reinjection. On the other hand reinjection of pregnant sensitized guinea-pigs rarely produced fatal shock. These results support the idea that the resistance to anaphylactic shock apparent in pregnancy may not be due to the prevention of the antigen-antibody reaction or its consequences but rather to the factors which lead to the formation of anaphylactic antibodies and to their being bound to the cells. The resistance shown by pregnant sensitized guinea-pigs and by the ones reinjected after parturition supports this theory and is born out by the figures in Table 1 and a comparison of those in Table 2 with those of Table 3.

On December 16, 1951, we sensitized 23 female guinea pigs of the same stock with 0.05 ml of normal horse serum. Eleven of these were pregnant and 12 virginal (Tables 2 and 3). After a period

TABLE 3

QUANTATIVE EXPERIMENTS ON THE REACTION OF VIRGINAL SENSITIZED GUINEA-PIGS TO INTRACARDIAL REINJECTION. THE GUINEA-PIGS WERE SENSITIZED WITH 0.05 ML NORMAL HORSE SERUM DILUTED UP TO 1 ML WITH PHYS. NACL SOLUTION ON DEC. 16, 1951. REINJECTED INTRACARDIALLY WITH THE QUANTITY OF HORSE SERUM GIVEN IN THE TABLE DILUTED TO 1 ML

No.	Date	Reinjection		Anaphylactic reaction
		Incubation in days	Antigen in ml	
1	Jan. 1, 1952	16 days	0.04	++
2	»	»	0.06	++
3	»	»	0.08	++
4	»	»	0.08	+++
5	»	»	0.10	+++
6	»	»	0.15	+++
7	Jan. 15, 1952	30 days	0.05	+
8	»	»	0.10	++
9	»	»	0.125	++
10	»	»	0.135	++
11	»	»	0.150	+++
12	»	»	0.200	+++

0 = No visible shock symptoms
\+ = Masseter convulsions or quite mild bronchospasms
++ = Severe bronchospasms and protracted shock
+++ = Shock leading to death

of either 16 or 30 days from the sensitization antigen was intracardially reinjected into the animals, diluted to one millilitre with physiological sodium chloride. All the pregnant guinea pigs had delivered before the reinjection. From Table 2 it appears that the minimum lethal dose for a pregnant sensitized guinea pig after an incubation period of 16 days was 0.6 ml and after 30 days it was 0.7 ml. For the virginal sensitized animals it was 0.08 after 16 days and 0.150 ml after 30 days from the sensitization. If we assume that the minimum lethal dose of antigen indicates the sensitiveness then a non-pregnant sensitized guinea pig acquires about six times as great sensitiveness, on an average, than a pregnant one. As we said in the introduction, a protection of this kind against the danger of sensitization, arising in pregnancy, seems a more reasonable thing than a protection against shock only after an antigen-antibody reaction.

SUMMARY

The writers studied the effect of pregnancy on anaphylactic shock in guinea-pigs by sensitizing both pregnant and non-pregnant animals with 0.5 ml of normal horse serum subcutaneously. The guinea-pigs were reinjected intracardially with an equal quantity of the same serum. It was then observed that the pregnant sensitized animals were much more resistant than the non-pregnant ones irrespective of whether the antigen was injected during the pregnancy or after the partus. The animals sensitized when non-pregnant but reinjected in the last half of pregnancy reacted to the reinjection like normal animals. If we take the minimum lethal dose of antigen as the measure of sensitiveness, an animal sensitized when pregnant and reinjected after partus is about six times less sensitive than one sensitized and reinjected in a virginal state.

REFERENCES

1. Ahlmark, A.: Acta Physiol. Scand. 1945:*9:*suppl. 28.
2. Cionini, A.: Pathologica 1927:*19:*478.
3. Cionini, A.: Boll. Ist. sieroter. milan. 1928:*7:*519.
4. Doerr, R.: Die Anaphylaxie I, Springer-Verlag, Wien 1950.
5. Hiller, E.: Zschr. ges. exp. Med. 1950:*115:*638.
6. Kellaway, C. H., and Cowell, J. S.: Brit. J. Exp. Path. 1922:*3:*268.
7. Lin, H.: Am. J. Obst. 1947:*54:*97.
8. Lumiere, A., and Couturier, H.: Compt. rend. Acad. sc., Par. 1921: *172:*772.
9. Pylkkö, O. O., and Vartiainen, A.: Acta pharm. tox., Kbh. 1947:*3:*97.
10. Raiman, L.: Science 1944:*106:*368.
11. Ratnoff, O. D.: Proc. Soc. Exper. Biol. 1939:*40:*248.
12. Solomonica, B., and Kurzrock, R.: Endocrinol. 1936:*20:*171.
13. Spanio, M., and Austoni, M.: Sperimentale 1941:*95:*747.
14. Thomsen, O.: Zschr. Immunforsch. 1917:*26:*213.
15. Werle, E., and Effkemann, G.: Arch. Gyn. Münch. 1940:*170:*82.

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A NEW REACTION FOR RHEUMATIC DISEASES

by

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There are several reactions by means of which it is possible to bring out differences between the sera of patients suffering from rheumatic diseases and normal serum. However, only a few of these are of greater significance in practice. Among them may be mentioned the Waaler-Rose reaction which, according to Wager (9), gives a positive result with rheumatoid arthritis in 61 per cent but is nevertheless not absolutely specific for this disease as 3 per cent of persons with rheumatic fever react positively, the corresponding figure for other diseases and healthy persons being 2 and 1 per cent respectively.

Weltmann coagulation reaction is considered by many investigators (8, 10, 11) an excellent indicator of the prognosis both in rheumatoid arthritis and rheumatic fever. The reaction is far from specific for these diseases.

According to some recent investigations (1, 2) the presence of C-reactive protein in the serum is regarded as the most sensitive standard of activity in rheumatic diseases.

The writer (5) has previously reported on a method by means of which the difference between the sera of patients suffering from rheumatoid arthritis and the sera of healthy persons can be demonstrated. This paper aims at investigating, by using approximately the same technique, a more extensive material of rheumatic diseases and at finding out whether the difference observed is characteristic of rheumatic diseases only.

TECHNIQUE

The method of procedure which differed somewhat from the one used in a previous work of the writer (5), was the following. 2.5 ml of 94 per cent ethyl alcohol was measured out into a test tube. 0.25 ml of the serum to be examined was pipetted into it at room temperature, a drop at a time, when a white precipitate formed. The test tube was put in the ice-box for the night. It was then centrifuged at 2500 r.p.m. for 5 minutes. The supernatant was poured off and the test tube was allowed to stand, turned upside down, at room temperature for 15 minutes. 1 ml of 95 per cent sulphuric acid was measured out into the tube onto the precipitate. After 5 minutes at room temperature the result was read. It was considered positive (+) if the precipitate had dissolved completely and a strong brown colour was produced; slightly positive (±) if the precipitate had dissolved partly and some brown colour was produced; and negative (—) if the precipitate had not dissolved and the sulphuric acid remained colourless. It was endeavoured all the time to keep the procedure exactly the same as far as possible as it appeared that changes in procedure may affect the final result even very considerably. Further, the sera were examined in fairly large series (about 10—60 tubes) which included, by way of control, at least two known sera giving a positive result of different strength. They were stored deep-frozen. If the same serum was examined more than once the average of the results obtained was taken as the final reading.

MATERIAL

The rheumatic material consisted of 42 sera obtained from adult hospital patients. 28 of them had rheumatoid arthritis and 14 rheumatic fever. The patients had one of the diseases mentioned as their only ailment, or their other diseases were such that they could not be considered to affect the results on the basis of the control material to be introduced later. None of them had been treated with cortisone or ACTH[1]. The investigation was performed in two successive days, not less than four times in all for each sample.

[1] The samples were from the Municipal Department of the Kivelä Hospital and Medical Clinic I and II, Helsinki University.

The sera of 129 healthy persons were examined as control material. They were obtained from unselected blood donors[1]. On these sera too the tests were carried out on two successive days not less than four times in all.

The majority of the sera examined consisted of samples sent to the Department of Serology and Bacteriology, University of Helsinki for serological routine tests, chiefly for syphilis reactions. They totalled 1263[2]. On sera which gave a positive reading the test was performed as often as the serum supply permitted, but in negative cases one test was sufficient. This was due to the fact that there was generally not much left of the sera as the greatest part had been used in the routine tests. This fact makes the evaluation of the results somewhat difficult, as will be apparent later. However, it would have been extremely difficult to obtain by another procedure such an extensive and varied material.

RESULTS

As regards the rheumatic material, Table 1 presents the proportion of the sera giving a positive and negative reading in different diseases.

TABLE 1

RESULTS IN VARIOUS RHEUMATIC DISEASES.

Diagnosis	Number of Cases	Positive		Negative	
		Number	Per Cent	Number	Per Cent
Rheumatoid arthritis	28	21	75	7	25
Rheumatic fever	14	9	64	5	36
Total	42	30	71	12	29

The dependence of the results on the sedimentation rate (Westergren's method) is shown in Table 2. It reveals that when the sedimentation rate was $\leqq$ 20 mm/hour all results were negative. The higher the sedimentation rate the greater the proportion of cases with positive reaction until all the cases became positive

[1] These sera came from the blood service of the Finnish Red Cross.

[2] The material covered all the clinics of the University of Helsinki, the Maria Hospital and the Municipal Medical Department of the Kivelä Hospital. However, the part concerned with skin diseases is not included in the present paper but will be published at a later date (7).

TABLE 2

THE EFFECT OF THE SEDIMENTATION RATE ON THE RESULTS

Sediment-ation rate	Rheumatoid Arthritis		Rheumatic Fever		Total	
	Posit.	Negat.	Posit.	Negat.	Posit.	Negat.
< 20....	—	2	—	1	—	3
20—30....	3	3	2	1	5	4
31—45....	4	2	2	3	6	5
> 45....	14	—	5	—	19	—
Total	21	7	9	5	30	12

starting from the value 45 mm/hour. The phenomenon was the same with both rheumatoid arthritis and rheumatic fever.

Also the results indicate dependence on fever (Table 3). Observations were made for a minimum of two days before and after the samples were taken. Feverish patients all gave a positive results and, on the other hand, all negative cases were without fever. True, it must be noticed that the latter group had three cases one of which registered once 37.3 C and two 37.2 C, but as the other readings were below 37.0 C these three cases have been regarded as having a normal temperature. With the patients who had fever the temperature varied greatly. The highest individual reading was 39.1 C.

It may be mentioned further that in rheumatic fever X-ray examination of the joints was made in five cases in the positive group. Four of them displayed changes caused by arthritis. On the negative side 3 cases were examined. All gave a negative results roentgenologically.

Judging by the present material the duration of the disease had no obvious effect on the results.

TABLE 3

THE EFFECT OF FEVER ON THE RESULTS

	Rheumatoid Arthritis		Rheumatic Fever		Total	
	Posit.	Negat.	Posit.	Negat.	Posit.	Negat.
Without fever ..	11	7	3	5	14	12
With fever	10	—	6	—	16	—
Total	21	7	9	5	30	12

TABLE 4

DEPENDENCE OF THE STRENGTH OF THE POSITIVE REACTIONS ON SEDIMENTATION RATE AND FEVER

Reading	Rheumatoid Arthritic						Rheumatic Fever					
	Number of Cases	SR			No Fever	Fever	Number of Cases	SR			No Fever	Fever
		20—30	31—45	> 45				20—30	31—45	> 45		
±	14	3	3	8	10	4	7	2	2	3	3	4
+	7	—	1	6	1	6	2	—	—	2	—	2

6 of the patients examined had been treated with salicylates 5 gave a positive results and 1 negative. The number of patients who had definitely not received, either salicylates or other pain-relieving or fever medicines was also 6. Three of them gave a positive, three a negative reaction. The dissolving of sodium salicylate in five positive and five negative sera up to a concentration of 0.1 mg/ml caused no change in the mode of reaction.

The positive cases have been analysed according to the strenght of reaction in Table 4. If we differentiate between slightly (reading ±) and strongly (reading +) positive cases we find that sedimentation generally is higher in the latter and that they show a greater number of feverish patients.

Sera from healthy persons gave a completely negative result in each case. There was not a single ± reading.

Of the samples, totalling 1263, sent for serological routine tests 90 or about 6.8 per cent gave a positiv result. There are no rheumatic cases in this section of the material. The samples were broken down between different special hospitals as follows:

Internal medicine	609	cases	of	which	62	positive	i.e.	10.2	per	cent
Surgical	293	»	»	»	14	»	»	4.7	»	»
Gynaecological and obstetrical	162	»	»	»	6	»	»	3.7	»	»
Pediatric	84	»	»	»	2	»	»	2.4	»	»
Psychiatrical and neurological.....	55	»	»	»	3	»	»	5.5	»	»
Radiotherapeutical	41	»	»	»	3	»	»	7.5	»	»
Eye diseases	13	»	»	»	—	»	»	—	»	»
Ear, nose and throat diseas.	6	»	»	»	—	»	»	—	»	»
Total	1263	cases	of	which	90	positive	i.e.	6.8	per	cent

The grouping of the positive results on the basis of the diagnosis is greatly hampered by the fact that the same patient may have several diagnoses. A part of these, however, were such as to make changes in the serum unlikely. In other cases the diagnosis adopted has been the one considered the patients principal diagnosis. The following list gives the proportion represented by the number of positive cases, as compared with the total in each instance (in the brackets are mentioned on each occasion the number of cases where it was possible to perform the test once only and in which a slightly positive results was arrived at):

So-called collagen diseases

Erythema nodosum	2/2	
Lupus erythematosus disseminatus	2/2	
Thromboangiitis obliterans	1/6	

Liver diseases and diseases of the bile duct

Cirrhosis of the liver	5/6	
Infectious hepatitis	5/7	(3)
Cholelithiasis	2/19	
Cholecystitis	2/18	

Diseases of the bone marrow and lymphatic tissue

Hodgkin's disease	4/6	
Monocytic leukaemia	1/2	
Myelogenous leukaemia	1/2	(1)
Myeloma	2/2	
Panmyelophthisis	1/2	

Kidney diseases

Congenital polycystic disease of the kidney	1/2	
Nephritis (acute or chronic)	5/12	(1)
Nephrosclerosis	1/7	
Tumor of the kidney	2/4	(1)

Infections

Abscess of the lung	1/1	
Acute parametritis	1/2	
Colitis	1/9	
Febris e causa ignota	1/3	
Pneumonia	1/25	
Tuberculosis	4/25	(2)
Acute appendicitis	1/33	(1)
Purulent mastitis	1/2	(1)
Subacute bacterial endocarditis	1/3	(1)
Thrombophlebitis	1/6	(1)
Tonsillitis	1/6	(1)

Pregnancies and their complications

Graviditas m. X	1/12	
Abortus m. II—III	2/9	
Nephropathia gravidarum	1/5	

Diseases of the thyroid gland

Nodular goiter	2/20	(1)
Thyrotoxicosis	3/26	

Carcinoma (all very far advanced cases)	10/62	(2)
Varicose ulcer	3/6	
Diabetes mellitus	3/26	(1)

In addition to the above-mentioned the following occur as sporadic diagnoses:

Angina pectoris	1/12	
Calcification of the pericardium	1/1	
Degenerative arthritis	1/7	
Hyperglobulinaemia	1/1	
Imbecility	1/2	
Myalgia	1/1	
Myocardial infarction	1/14	
Senile gangrene	1/1	
Chronic alcoholism	1/3	(1)
Epilepsy	1/9	(1)

Melena	1/6 (1)
Neurocirculatory asthenia	1/22 (1)
Peptic ulcer	1/64 (1)

Of the cases giving a positive result only 3 registered a sedimentation rate $\leq$ 10 mm/hour. In all other cases it was higher.

DISCUSSION

An examination of the results shows that about 71 per cent of all rheumatic cases reacted positively and that there was no great difference between rheumatoid arthritis and rheumatic fever in this respect. Normal sera all reacted negatively. The results thus resemble more those obtained by Weltmann coagulation reaction (11) than the ones obtained by Waaler-Rose reaction (9).

The dependence of the results on the activity of the process of the disease is proved by the fact that mild cases react negatively and the more serious the cases in question the greater becomes the proportion of positive readings untill the 100 per cent mark is reached from a certain limit.

The effect of salicylates on the results could not be observed on the basis of the above, admittedly rather limited material as far as therapeutic serum concentrations are concerned. The results obtained by Weltmann coagulation reaction have also been ascertained to be independent of the salicylate content of the serum (10). On the other hand salicylates have a distinctly reducing effect on the sedimentation rate (3), especially in cases where the rate is increased.

Positive results have been arrived at in many other besides rheumatic diseases, the majority being diseases in which it is known that the serum globulins generally increase (6, 11). This supports the assumption that the proportionate increase of serum globulins would be at least one appreciable reason for the positive reaction. This increase, in turn, would cause changes in the precipitating and dissolving qualities of proteins. Giving a similar indication is the observation of the writer (5) that cortisone treatment changes the positive mode of reaction to a negative direction since it has been found that the administration of cortisone has a reducing effect on serum globulins (4).

With regard to the positive results of the last part of the material, it should be noted that it was possible in many cases to perform the reaction once only. As was already pointed out in discussion of the technique, even small deviations may change the final result. Although an effect has been made all along to keep the technique exactly the same as much as possible, there is reason on the basis of the above to adopt a somewhat conservative attitude to the results in individual cases in which the investigation has been performed only once and a slightly positive result been arrived at.

SUMMARY

1. A procedure has been described by means of which serum gives in rheumatic diseases a positive result in about 71 per cent.

2. Healthy persons react 100 per negatively.

3. The positive nature of the results is dependent on the activity of the diseases.

4. The positive result is not specific for rheumatic diseases.

REFERENCES

1) Anderson, H. C., and Mc Carty, M.: Am. J. Med. 1950:*8:*445.
2) Hill, A. G. S.: Lancet 1951:*261:*807.
3) Homburger, F.: Am. J. Med. Sci. 1945:*210:*168.
4) Jager, B. V., Brown, H., and Nickerson, M.: J. Lab. Clin. Med. 1951:*37:*431.
5) Jokinen, E. J.: Ann. Med. Exp. Biol. Fenn. 1952:*30:*72.
6) Kagan, B. M.: Am. J. Med. Sci. 1943:*206:*309.
7) Pätiälä, R., and Jokinen, E. J. (to be published).
8) Scherlis, S., and Levy, D. S.: Am. Heart J. 1943:*26:*355.
9) Wager, O.: Ann. Med. Exp. Biol. Fenn. 1950:Suppl. No. 8:28.
10) Ward, D. E. Jr, and Harrell, G. T.: Am. J. Med. Sci. 1946:*211:*157.
11) Wuhrmann, F., and Wunderly, Ch.: Die Bluteiweisskörper des Menschen. Benno Schwabe & Co. Basel 1947.

AUS DEN KLINIKEN DER UNIVERSITÄT TURKU: INNER-MEDIZINISCHE KLINIK UND KINDERKLINIK

NEUE BEOBACHTUNGEN AM PRESSORISCHEN KÄLTETEST

von

M. K. PAASONEN, E. K. WARIS und T. E. PELTONEN

(Eingegangen am 8. September 1952)

Zur Bestimmung der Empfindlichkeit der Blutdruckveränderungen sind vielerlei Teste entwickelt worden, mit denen man auf diese Blutdruckveränderungen in der einen oder andern Richtung einzuwirken versucht. Aus der sich dabei zeigenden Reaktion will man dann bestimmte Schlussfolgerungen über die Beschaffenheit des Blutkreislaufsystems ziehen. Damit ein solches Verfahren als zuverlässig angesehen werden darf, müssen natürlich der Reiz und die Versuchsbedingungen so konstant wie möglich gehalten werden. 1932 wurde für diesen Zweck von Hines u. Brown (6.) der sogen. Pressorische Kältetest (PKT) vorgeschlagen. Die praktische Durchführung des PKTs beschreiben wir weiter unten. Später hoben die genannten Forscher dessen Bedeutung für die Erkennung des sogen. prähypertensiven Zustandes hervor (4, 5.). Je nach der Reaktionsweise der Versuchspersonen ist ihr Material in drei Gruppen gegliedert. Personen mit normalem Blutdruck, deren Blutdruckanstieg beim PKT für den systolischen Druck unter 20 mm Hg und für den diastolischen Druck unter 15 mm Hg blieb, galten als normotonische Hypo- oder Normoreaktoren. Die Normotoniker mit einer PKT-Reaktion von mindestens 20 mm Hg systolisch und mindestens 15 mm Hg diastolisch galten als Hyperreaktoren. Die Hypertoniker reagierten auf den Kältereiz stärker als Personen mit normalem Blutdruck. Die Patienten mit arteriosklerotischer Hypertonie reagieren nicht so stark wie die mit essentieller Hypertonie, doch gehören auch sie zu den Hyperreaktoren oder befinden sich an der oberen Grenze des normalen Bereichs. Arteriosklerose

für sich allein steigert die PKT Empfindlichkeit nicht. Bei einer auf chronischer Nierenkrankheit beruhenden Hypertonie ist der Pressorische Kältereflex im Durchschnitt normal [Hines u. Brown (7.) Miller u. Burger (9.)] Hammarström (3.) betont jedoch, dass sich der PKT für die Differentialdiagnose von essentieller und renaler Hypertension nicht verwenden lasse, weil die Schwankungen zu gross seien.

In einer späteren Arbeit haben Hines u. Brown (8.) gezeigt, dass die PKT-Reaktion eine individuelle Eigenschaft ist, die sich Jahre hindurch unverändert erhalten kann. Russek u. Zohman (12.) behaupteten, dass die PKT-Reaktion der Normotoniker mit dem Alter zunehme. Auch bei Hines u. Brown (8.) waren die Reaktionen der Personen über 40 Jahren stärker als die der jüngeren Altersklassen. Miller u. Burger (9.) konnten keine nennenswerten Unterschiede zwischen den verschiedenen Altersklassen feststellen.

Garai (2.) beobachtete eine Einwirkung des Tonus der peripheren Blutgefässe auf die PKT-Reaktion: in der warmen Hand ist der Tonus niedrig und bleibt der Blutdruckanstieg gering; im kalten Glied umgekehrt.

Hammarström (3.), Pickering u. Kissin (10.) haben gefunden, dass die PKT-Reaktion im allgemeinen nicht grösser ist als die Reaktion auf einen beliebigen zufälligen Reiz, sowie dass die Tages kurve oft grössere Blutdruckanstiege zeigt, als wie sie der PKT auslöst.

Bei uns hat Brummer (1.) Pressorische Kältereflexe bei Dystonia neurocirculatoria untersucht, mit dem Ergebnis dass Hyperreaktion bei diesen Dystonikern nicht häufiger auftritt als im Vergleichsmaterial. Falls der PKT als Massstab für den prähypertensiven Zustand angesehen werden darf, so würde dies bedeuten, dass die Dystoniker nicht mehr für Hypertonie disponiert sind als andere Menschen.

Die im Schrifttum dargestellten PK-Teste sind durchweg »in the classic way» von Hines u. Brown durchgeführt worden, bei dem sich der Patient liegend in völliger Ruhe befindet, solange bis wiederholte Blutdruckmessungen einen konstanten Wert zeigen. Darauf wird die eine Hand 1 Minute lang in Wasser von 0—4° gehalten und auch währenddessen und anschliessend der Blutdruck verfolgt, bis dessen Höhe wieder den Ausgangswert oder einen neuen festen Wert angenommen hat. Auch im Sitzen ist der Test durchgeführt worden.

EIGENE UNTERSUCHUNGEN

Wir haben die Teste sonst auf die eben beschriebene Weise vorgenommen, nur dass wir uns eines lose an den Unterarm gebundenen Plastikbeutels bedienten, der mit Wasser von 3.5—4° gefüllt war. Zur Konstanthaltung der Temperatur wurden Eisstücke nachgegeben. So konnte der Verssuch praktisch-gesprochen ohne jegliche Muskelbewegungen des Patienten ausgeführt werden. Gleichzeitig bemühten wir uns um einen möglichst ruhigen äusseren Rahmen für den Test. Als Versuchspersonen dienten gesunde Medizinstudenten, in der Genesung befindliche Kinderpatienten, sowie eigens für diesen Zweck ausgesuchte Hypertoniker, Dystoniker und als Vagotoniker betrachtete Ulcus-Patienten. Der Blutdruck wurde gemessen mit dem Tonometer in Verbindung mit einem Quecksilberbarometer.

Wir haben an 120 Versuchspersonen 192 PK-Teste vorgenommen. Unser Material ist verhältnismessig gering; trotzdem sind bei ihm verschiedene Einzelzüge hervorgetreten, die wir im bisherigen Schrifttum nicht angetroffen haben.

1. INVERSE REAKTION

Bei fünf Versuchspersonen (Tabelle 1.) hat sich beim PKT anstelle des üblichen Blutdruckanstiegs ein Absinken des Blutdrucks gezeigt. Eine Prüfung der Diagnosen ergab, dass bei diesen Patienten die seelische Komponente einen massgeblichen Anteil an der Krankheit hatte.

TABELLE 1

Alter	Gschl.	Diagnose	Blutdruck			Puls	
			in Ruhe	beim PKT	Veränderung	Ruhe	PKT
51	femin.	Neurose (Klimakt.)	210/120	180/110	—30/—10	100	110
14	femin.	Neurasthenie	145/75	135/65	—10/—10	92	104
34	masc.	Neurose Neuralgien	135/80	130/ 85	— 5/+ 5	66	70
45	femin.	Neurasthenie	155/100	145/100	—10/0	90	90
31	femin.	Dystonie	145/ 90	140/100	— 5/+10	60	64

2. REAKTIONEN DER VAGOTONIKER

Da es nahe liegt zu erwarten, dass der Tonus des vegetativen Nervensystems mitbeteiligt sei am Zustandekommen der PKT-Reaktion, versuchten wir diesem Zusammenhang nachzugehen durch Vornahme des Testes an Personen, in deren Status sich ein Überwiegen des Vagotonus hatte feststellen lassen. Als solche prüften wir 7 Ulcus-Patienten, 2 Dystoniker und 14 gesunde Personen. Nach der Pulsdichte gliederte sich die Gruppe folgendermassen:

Bis zu	80	Schläge	i.	Min.:	1
» »	70	»	»	» :	2
» »	60	»	»	» :	19
weniger als	50	»	»	» :	1

Der systolische Druck zeigte einen Anstieg um weniger als 5 mm Hg bei 14 Personen, um bis zu 10 mm Hg bei 3, und um bis zu 15 mm Hg bei 6 Personen. Der Anstieg des diastolischen Druckes war bei 15 Personen dem des systolischen Druckes gleich, bei den übrigen lag er bis zu 5 mm Hg niedriger als dieser. Die Vergleichsgruppe bestand aus 27 gesunden Personen mit normalem Blutdruck, die keine deutlichen Symptome einer Vagotonie aufwiesen. Bei ihr wechselte der Anstieg des systolischen Drucks zwischen 5 und 15 mm Hg, während der diastolische Druck bei 12 Personen im gleichen Masse anstieg, bei 15 Personen jedoch in geringerem Masse. Diese beiden Gruppen scheinen also keinen grundsätzlichen Unterschied in ihrer Reaktion auf den PKT zu zeigen.

3. VERGLEICH ZWISCHEN PRESSORISCHE KÄLTEREFLEXEN AM ARM UND AM BEIN

Da eine grosse Bedeutung für den PKT-Wert dem subjektiven Schmerzgefühl zuzukommen scheint (Wolf u. Hardy) (14.), wird man es für gleichgültig halten, an welcher Körperstelle das betr. Schmerzgefühl erzeugt wird. Wolff (13.) hat in seiner Arbeit die PKT-Reaktionen dreier Personen am linken und rechten Arm und auch am linken und rechten Bein miteinander verglichen. Zu fehlen schien es im Schrifttum jedoch an Vergleichen zwischen Arm und Bein. So haben wir bei 15 Personen im Alter von 10—60 Jahren nacheinander einen PKT am Arm und am Bein vorgenom-

men. In einem Teil der Fälle ging der Armtest, in einem andern Teil der Fusstest zeitlich voran, damit die Wirkung einer etwaigen Gewöhnung ausgeschaltet werde. 10 von den Versuchspersonen waren gesund, bei 3 war Dystonia neurocirculatoria festgestellt worden und bei 2 Hypertonia essentialis. Bei der Durchführung der PKTs achteten wir auf gleiche Grösse der dem Kältereiz ausgesetzten Hautfläche am Arm und am Bein. Eine Durchsicht der Reaktionswerte ergab, dass diese in 7 Fällen für Arm und Bein ganz gleich waren. Eine Verschiedenheit im Anstieg des systolischen Drucks lag bei 5 Personen vor: der Druckanstieg beim Arm war in einem Fall um 10 mm Hg grösser als beim Bein, in drei Fällen um 5 mm grösser und in einem Fall um 5 mm kleiner. Ebenso zeigte sich bei 5 Personen (obwohl nicht immer den gleichen) eine Verschiedenheit im Anstieg des diastolischen Drucks, und zwar um ganz entsprechende Betrage. Die Zahlen sind mit Rücksicht auf die möglichen Messungsungenauigkeiten auf den nächstgelegenen 5 mm-Vêrt abgerundet worden. Vergleichen wir diese Abweichungen mit denen der nacheinander am gleichen Glied gemessenen Werte, so sind beide gleicher Grössenordnung, sodass PKTs vom Arm und vom Bein im Bedarfsfall einander vertreten dürfen.

ZUSAMMENFASSUNG

Das Material umfasst 192 Pressorische Kälteteste an 120 Versuchspersonen. Unter diesen befinden sich 5 psychisch labile Personen, die einen negativen PKT-Wert gezeigt haben. Ein Vergleich zwischen Vagotonikern und »normalen» Individuen hat keine nennenswerten Unterschiede erkennen lassen. An welchem Körperglied der PKT vorgenommen wird, scheint gleichgültig zu sein.

LITERATUR

1. Brummer, P.: Ann. med. int. fenn. 1947:*36:*405.
2. Garai, O.: Brit. Heart. J. 1945:*7:*200.
3. Hammarström, S.: Acta med. scand. 1947, suppl. 192.
4. Hines, E. A.: Am. Heart J. 1940:*19:*408.
5. Hines, E. A.: J.A.M.A. 1940:*115:*271.
6. Hines, E. A., und Brown, G. E.: Proc. Mayo Clin. 1932:*7:*332.

7. Hines, E. A., und Brown, G. E.: Ann. Int. Med. 1933:*7:*209.
8. Hines, E. A., und Brown, G. E.: Am. Heart J. 1936:*11:*1.
9. Miller, J. H., und Burger, M.: Am. Heart J. 1939:*18:*329.
10. Pickering, G. W., und Kissin, M.: Clin. Sc., London 1935:*2:*201.
11. Russek, H. J.: Am. Heart J. 1943:*26:*398.
12. Russek, H., und Zohman, B.: Am. Heart J. 1945:*29:*113.
13. Wolff, H. H.: Q. J. Med., Oxf. 1951:*20:*261.
14. Wolf, S., und Hardy, J. D.: J. Clin. Invest. 1941:*20:*521.

AUS DEN KLINIKEN DER UNIVERSITÄT TURKU: KINDERKLINIK UND INNERMEDIZINISCHE KLINIK

VERSUCHE MIT DEM PRESSORISCHEN KÄLTETESTE IN ORTHOSTATISCHER LAGE

von

T. E. PELTONEN, E. K. WARIS und M. K. PAASONEN

(Eingegangen am 8. September 1952)

Nachdem Hines u. Brown (2, 3) in ihren Untersuchungen, zu dem Ergebnis gelangt sind, dass dem von ihnen entwickelten pressorischen Kältetest (PKT) eine bestimmte Bedeutung für die Erkennung des prähypertensiven Zustandes innewohnt — ist von den verschiedensten Seiten versucht worden, die Faktoren zu bestimmen, durch die Veränderungen in der PKT-Reaktion hervorgerufen werden können. Einige dieser Arbeiten haben auf solchem Wege versucht in den Mechanismus der Hypertonie einzudringen, meist lag jedoch das Ziel lediglich in einer Aufhellung des physiologischen Mechanismus des PKT.

Obwohl Reiser u. Ferris (5) geschlossen haben, dass auch die humorale Druckstrahlung am PKT-Ergebnis Anteil habe, indem sie bei einem Teil der mit Tetraäthylammoniumbromid behandelten Versuchspersonen eine positive Reaktion hervorrufe, so hat sich doch im allgemeinen die Auffassung durchgesetzt, dass die Drucksteigerung durch eine reflektorische Reizung des Blutdruckzentrums bewirkt wird. Gleich welche Bahn in diesem Reflexbogen der afferente Impuls durchläuft und von wo er herkommt, so muss er doch offenkundlich in das (oder die) vasokonstriktorische Zentrum (oder Zentren) gelangen. Wenn die Versuchsperson steht, so kann der Tonus ihres vasomotorischen Zentrums als erhöht gegenüber dem Ruhetonus angesehen werden. Demgemäss dürfen wir

erwarten, dass der PKT-Wert verschieden ausfallen wird, jenachdem ob der Test im Liegen oder im Stehen durchgeführt worden ist.

Im Schrifttum haben wir keinen in Orthostase durchgeführten PKT-Versuch genannt gefunden.

EIGENE UNTERSUCHUNGEN

Für die praktische Durchführung des PKTs verweisen wir auf die in unserm früheren Aufsatz gegebene ausführliche Darstellung (4). Den Übergang von der horizontalen zur orthostatischen Lage haben wir mittels eines Kipptisches vorgenommen. Zur Feststellung der Einwirkung der Orthostase auf die PKT-Reaktion haben wir an 38 Versuchspersonen insgesamt 98 PK-Teste vorgenommen. Das Alter der Personen schwankte von 5 bis zu 60 Jahren, nach dem Geschlecht teilten sie sich in 13 Frauen und 25 Männer. Das Material haben wir dann aufgegliedert in drei Gruppen, je nachdem ob die orthostatische Reaktion gegenüber der horizontalen grösser, gleichgross oder kleiner war.

1. Beide Reaktionen gleichgross

Gleichgross waren die Reaktionen bei 10 Personen, nämlich 7 Männern und 3 Frauen. Davon waren 6 gesund, bei 3 lautete die Diagnose auf Dystonia neurocirculatoria und bei 1 auf Neurosis vegetativa. Nach den im Liegen durchgeführten Testen besassen sie alle einen normalen Blutdruck und gehörten alle der normoreaktorischen Klasse nach Hines u. Brown (2) an. Eine orthostatische arterielle Anämie (OAA) zeigten 4 Personen. Als Kriterium verwandten wir hierfür u.a. nach Åkeson (7) die Steigerung des Pulses um mindestens 27 Schläge in der Minute beim Übergang aus horizontaler in orthostatische Lage. Bei den Versuchspersonen dieser Gruppe hat sich kein registrierbarer Unterschied in den PK-Testen gezeigt, die erst im Liegen durchgeführt waren und dann im Stehen (von mindestens 10 Min. Dauer).

2. *Reaktion im Stehen grösser als im Liegen*

Die Gruppe umfasst 9 Personen, davon 6 Männer und 3 Frauen. Gesund waren 4, dystonisch 5. Der Blutdruck war bei allen normal. Das Ergebnis ist aus der folgenden Übersicht zu erkennen:

PKT im Liegen	PKT im Stehen	OAA
7 normale Reaktion	4 Hyperreaktion 3 normale Reaktion	5
2 Hyperreaktion	2 Hyperreaktion	1

3. *Reaktion im Stehen kleiner als im Liegen*

Die dritte Gruppe umfasst 19 Personen, nach dem Geschlecht gegliedert in 12 Männer und 7 Frauen, nach dem Tonus in 5 Gesunde, 5 Hypertoniker und 9 Dystoniker. Die Dystoniker und die Gesunden hatten normalen Blutdruck. Die Hypertoniker dieser Gruppe hatten sich bei PKT im Liegen normoreaktorisch gezeigt. An ihnen wurden 8 PKTs im Stehen vorgenommen, von denen 5 ein Sinken des Blutdrucks um 15—25 mm Hg bewirkten (Hyporeaktion). Bei 1 Reaktion stieg der Blutdruck während der ersten halben Minute (des PKTs im Stehen) um 5 mm Hg an, fiel dann aber um 15 mm ab. Ebenso wurde bei den 2 letzten Reaktionen ein Anstieg um 10 mm registriert, aber schon zu Ende der kalten Minute ein Absinken um 15 und 20 mm. Die Reaktionen der übrigen Personen dieser Gruppe stellt die folgende Übersicht dar:

PKT im Liegen	PKT im Stehen	OAA
10 normale Reaktion	5 normale Reaktion 5 Hyporeaktion	5
4 Hyperreaktion	1 Hyperreaktion 3 normale Reaktion	2

DISKUSSION

Die orthostatische Belastung wirkt sich bei den verschiedenen Menschen ganz individuell aus. Die oben dargestellten Teste sind nach 10 Minuten Stehen durchgeführt worden, bei anderer Zeitdauer des Stehens wären die Ergebnisse natürlich etwas anders ausgefallen. Es scheint, dass zu Anfang der Orthostasie der PKT-Wert ansteigt. Bei einigen Menschen dauert diese Phase sehr lang (bis zu mehreren zehn Minuten), bei andern dagegen nur ganz kurz

(weniger als eine Minute). Danach nimmt, wenn die Orthostase fortdauert, der PKT-Wert ab. Die orthostatische arterielle Anämie und eine Hyporeaktion oder inverse Reaktion sind nicht aneinander gebunden, denn in unsern Versuchen konnte eine Hyporeaktion während des PKTs auch ohne orthostatische Hypotension auftreten. Wenn dagegen der systolische und gleichzeitig der Pulsdruck des Patienten während der orthostatischen Belastung abnehmen, so scheint der PKT dieses Nachlassen des Druckes zu beschleunigen, jedenfalls aber nicht aufzuhalten, und der Patient kann in Kollaps geraten. Ist der Zustand schon so kritisch, dass das Blutdruckzentrum die vom Stehen bewirkte Belastung nicht mehr zu kompensieren vermag, sondern ein Kollaps droht, so kann die auf das gleiche Zentrum gerichtete zusätzliche Belastung durch den PKT den Kollaps auslösen. Wir finden hier eine Parallele zu der Beobachtung Baylis', dass die Pressorreflexe bei Chloroformanästhesie einen depressorischen Charakter bekommen (1). (Das Chloroform übt ja eine entschieden lähmende Wirkung auf die vasomotorischen Zentren aus.) Bei Nitritsynkope, wenn wir es jetzt mit dem orthostatischen Kollaps vergleichen, Adrenalin und andere Mittel, die den Arteriolenspasmus verursachen, den Zustand nicht bessern, sondern vielmehr verschlechtern (6). Auch lässt sich die Wirkung des Kältereizes so denken, dass er durch Verengerung der Arteriolen den Blutkreislauf herabsetzt und die Anoxie des Gewebes steigert.

ZUSAMMENFASSUNG

Untersucht wurde die Einwirkung der orthostatischen Lage auf den pressorischen kältetest bei 38 Personen. Diese Einwirkung scheint ganz individuell zu sein. Es sieht danach aus, dass der Übergang vom Liegen zum Stehen zunächst den PKT-Wert erhöht, dass ihn aber die fortdauernde orthostatische Belastung herabmindert. Die von der Orthostase bewirkten Veränderungen des PKT-Wertes waren nicht gebunden an die orthostatische arterielle Anämie. Im präkollaptischen Zustand schien der PKT das Absinken des Blutdrucks wenigstens nicht aufzuhalten, eher sogar zu beschleunigen.

LITERATUR

1. Baylis, W. M.: Proc. Roy. Soc. 1908:*B 80:*339, zit. von Best, C. H. & Taylor, N. B.: The Physiological Basis of Medical Practice, Balliere, Tindal & Cox, London 1950.
2. Hines, E. A.., und Brown, G. E.: Proc. Mayo Clin. 1932:*7:*332.
3. Hines, E. A., und Brown, G. E.: Ann. Int. Med. 1933:*7:*209.
4. Paasonen, M. K., Waris, E. K., und Peltonen, T. E.: Ann. exp. med. biol. fenn. in press..
5. Reiser, M. F., und Ferris, E. B.: J. Clin. Invest. 1948:*1:*156.
6. Wilkins, R. W., Weiss, S., und Haynes, F. W.: J. Clin. Investig. 1938:*17:*41.
7. Åkeson, S.: Upsala Läkarför. Förh. N. F. 1936:*41:*383.

AUS DEM ANATOMISCHEN INSTITUT DER UNIVERSITÄT HELSINKI UND DEM WIHURI-FORSCHUNGSINSTITUT ZU HELSINKI

ÜBER DIE WIRKUNG VON DIGITALIS UND METHYLTHIOURAZIL AUF DIE SCHILDDRÜSE BEI GLEICHZEITIGER ZUFUHR

von

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Bei der Behandlung von Fällen schwerer Herzinsuffizienz, wo die zentrale Kreislauffunktion bereits dermassen herabgesetzt ist, dass die üblichen Behandlungsmethoden nicht mehr zur Herstellung des Gleichgewichts ausreichen, hat man versucht durch Verminderung des Gesamtstoffwechsels eine Entlastung des Herzens zu erreichen. Zwei Wege können zu diesem Zweck beschritten werden: Zerstörung oder Entfernung eines möglichst grossen Teiles der Schilddrüse, oder Verminderung ihrer Sekretion. Seitdem die Antithyreoideastoffe bekannt wurden, hat man versucht, diese bei der Behandlung von Fällen mit Herzinsuffizienz zu verwenden, und somit die gefährlichere operative Behandlung zu vermeiden. Die Antithyreoideastoffe haben die Eigenschaft, in eine gewisse Phase der Synthese des Schilddrüsenhormons verhindernd einzugreifen. Das aktive Hormon wird somit nicht von der Drüse ausgeschieden, und der Gesamtstoffwechsel wird herabgesetzt. Parallel dieser Wirkung bedingen die Antithyreoideastoffe eine Hyperplasie der Schilddrüse mit Kropf, was nach heutigem Wissen durch das Fehlen des physiologischen Inhibitors der Thyreotropinsekretion durch die Hypophyse, des Schilddrüsenhormons, zu erklären ist. Über die Wirkung von Digitalis auf die Schilddrüse wissen wird, dass kleine Mengen bei Versuchstieren den durch vorhergehende Thyreotropinzufuhr gesteigerten Gesamtumsatz herabsetzen (3). Kuusisto (6)

hat in einer früheren Arbeit eine inaktivierende Wirkung der Digitalisglykoside auf das histologische Bild der Schilddrüse nachgewiesen. Das Drüsenepithel wurde niedriger unter gleichzeitiger Zunahme des Kolloids. Weiterhin schien die Aktivität der einzelnen Epithelzellen vermindert.

Die Verfasser haben früher gezeigt (4), dass das k-Strophantin die Metamorphose bei Kaulquappen verzögert, und den Zuwachs derselben beschleunigt, was auf eine herabgesetzte Schilddrüsenfunktion hinweist. Bei Fällen schwerer Herzinsuffizienz, wo der Artzt durch Antithyreoideastoffe den Gesamtumsatz des Patienten herabgesetzt hat, ist trotzdem eine Verabreichung von Digitalispräparaten nötig.

Es wäre deshalb von besonderem Interesse zu wissen, wie sich eine gleichzeitige Zufuhr von Antithyreoideastoffen (gewöhnlich verschiedene Derivate des Thiourazils) und von Digitalispräparaten auf die Schilddrüse auswirkt. Eine Untersuchung über die zusammengeordnete Wirkung von Thiourazil und Digitalis auf das histologische Bild der Schilddrüse bei Versuchstieren schien uns deshalb erwünscht.

MATERIAL UND METHODIK

Die Versuche wurden an 30 männlichen Meerschweinchen gleichen Stammes ausgeführt. Die Versuchsbedingungen waren für sämtliche Tiere gleich. Drei Versuchsreihen, jede 10 Tiere umfassend, wurden durchgeführt. Es wurde die Wirkung von Methylthiourazil (MTU) und einer Mischung der Lanataglykoside, Digilan (Digilan Orion), auf die Schilddrüse untersucht. Das Methylthiourazil wurde durch Katheter in Wasserlösung in den Magen eingeführt, das Digilan als intraperitoneale Injektion in 20 vol. %-iger alkoholischer Lösung gegeben. Die Meerschweinchen wurden durch Aethernarkose getötet. Nach Entfernung wurden die Schilddrüsen gewogen und in Helly's Lösung während 2 Stunden fixiert. Nach Waschen wurden die Präparate in einer Serie von Aethyl—n-butylalkohol entwässert und in Paraffin eingebettet. Von jedem Lappen wurden an verschiedener Stelle vier Schnitte hergestellt. Die Präparate wurden nach Bensley (2) gefärbt. An den Präparaten wurden nach dem Verfahren von Uotila und Kannas (8) die prozentuellen Mengen von Epithel, Kolloid und Stroma gemessen.

VERSUCHE UND ERGEBNISSE

In der ersten Versuchsreihe wurde während drei Wochen fünf Meerschweinchen täglich 0.1 g MTU sowie intraperitoneal Kontrollösung (20%-ige alkoholisches Lösungmittel für Digilan) pro Tier gegeben. Den übrigen fünf Meerschweinchen wurde pro Tier 0.2 mg/kg Digilan und 0.1 g MTU täglich zugeführt.

In der zweiten Versuchsreihe wurde zuerst fünf der Tiere während eines Monats 0.2 mg/kg Digilan täglich zugeführt, während den übrigen fünf Tieren die Kontrollösung gegeben wurde. Nach Verlauf eines Monats wurde allen Versuchstieren der Reihe noch zusätzlich 0.05 g MTU gegeben, und zwar während zwanzig Tagen.

Die dritte Versuchsreihe unterscheidet sich von der zweiten nur durch die kleinere MTU-Dosierung, nämlich 0.025 täglich pro Tier. In der letzten Versuchsreihe starben ein MTU-Tier an Oesophagusperforation, und ein Digilan & MTU-Tier an Pneumonie. Diese Tiere wurden nicht bei der Beurteilung der Ergebnisse mitgerechnet.

Bei der Sektion war makroskopisch kein regelmässiger Unterschied zwischen den verschiedenen Gruppen festzustellen. Histologie der Schilddrüsen: Bei sämtlichen untersuchten Objekten war das histologische Bild der Schilddrüse ausgesprochen aktiv. Das Epithel war hoch, zylindrisch und prismatisch, die Form der Follikel unregelmässig sternförmig oder geschrumpft. Kolloid lag sehr spärlich vor, oft war in den spaltförmigen Follikellumina nur ein ungefärbter Schaum zu sehen. Durch die mikroskopische Untersuchung konnte kein klarer Unterschied in den Schilddrüsen aus den verschiedenen Gruppen festgestellt werden. Möglicherweise lag bei den Schilddrüsen der MTU & Digilan-Gruppen eine noch grössere Aktivität als bei den mit reinem Thiourazil behandelten vor.

Aus den Tafeln 1—6 sind Gewicht der Tiere vor und nach der Behandlung, Gewichtsveränderungen in Prozent, Gewicht der Schilddrüse auf 100 g Körpergewicht gerechnet, sowie prozentuale Zusammensetzung der Schilddrüse aus Epithel und Kolloid, ersichtlich.

TAFEL 1

MEERSCHWEINCHEN NACH TÄGLICHER PERORALER ZUFUHR VON 0.1 G MTU UND INTRAPERITONEALER ZUFUHR DER KONTROLLÖSUNG WÄHREND 21 TAGEN

Versuchstier Nr.	Gewicht zu Beginn des Versuchs	Gewicht nach Beendigung des Versuchs	Gewichtsveränderung	Gewicht der Schilddrüse	Epithel	Kolloid
1.	440 g	375 g	—14.8 %	20.0 mg/100 g	79.5 %	11.5 %
3.	495 »	415 »	—16.1 »	12.8 »	65.0 »	23.0 »
5.	370 »	310 »	—16.1 »	16.8 »	78.0 »	7.5 »
7.	470 »	455 »	— 3.2 »	11.9 »	70.0 »	21.0 »
9.	440 »	435 »	— 1.1 »	11.9 »	77.5 »	16.0 »
Mittelwerte	443 g	398 g	—10.3 %	14.7 ± 1.6	74.0 ± 2.8	15.8 ± 2.9

TAFEL 2

MEERSCHWEINCHEN NACH TÄGLICHER ZUFUHR VON 0.1 G MTU PERORAL UND 0.2 MG/KG DIGILAN INTRAPERITONEAL WÄHREND 21 TAGEN

Versuchstier Nr.	Gewicht zu Beginn des Versuchs	Gewicht nach Beendigung des Versuchs	Gewichtsveränderung	Gewicht der Schilddrüse	Epithel	Kolloid
2.	440 g	365 g	—17.0 %	8.9 mg/100 g	77.5 %	16.0 %
4.	420 »	345 »	—17.8 »	14.5 »	77.0 »	6.0 »
6.	410 »	380 »	— 7.3 »	15.0 »	68.0 »	12.0 »
8.	370 »	350 »	— 5.4 »	16.3 »	79.0 »	11.0 »
10.	460 »	490 »	+ 6.5 »	28.6 »	78.5 »	13.0 »
Mittelwerte	420 g	386 g	— 8.1 %	16.6 ± 3.2	76.0 ± 2.0	11.6 ± 1.9

TAFEL 3

MEERSCHWEINCHEN NACH 30-TÄGIGER INTRAPERITONEALER ZUFUHR DER KONTROLLÖSUNG UND NACHFOLGENDER PERORALER VERABREICHUNG VON ZUSÄTZLICH 0.05 G MTU TÄGLICH WÄHREND 20 TAGEN

Versuchstier Nr.	Gewicht zu Beginn des Versuchs	Gewicht nach Beendigung des Versuchs	Gewichtsveränderung	Gewicht der Schilddrüse	Epithel	Kolloid
11.	475 g	540 g	+13.7 %	33.0 mg/100 g	66.5 %	9.5 %
13.	500 »	545 »	+ 9.0 »	22.2 »	70.5 »	4.5 »
15.	425 »	505 »	+18.8 »	23.6 »	75.5 »	4.5 »
17.	455 »	550 »	+20.8 »	26.0 »	70.0 »	7.5 »
19.	535 »	620 »	+15.9 »	22.9 »	71.5 »	11.5 »
Mittelwerte	476 g	552 g	+15.6 %	25.5 ± 1.9	70.8 ± 1.5	7.7 ± 1.6

TAFEL 4

MEERSCHWEINCHEN NACH 30-TÄGIGER INTRAPERITONEALER ZUFUHR VON 0.2 MG/KG DIGILAN TÄGLICH UND NACHFOLGENDER ZUSÄTZLICHER PERORALER ZUFUHR VON TÄGLICH 0.05 G MTU WÄHREND 20 TAGEN

Versuchstier Nr.	Gewicht zu Beginn des Versuchs	Gewicht nach Beendigung des Versuchs	Gewichtsveränderung	Gewicht der Schilddrüse	Epithel	Kolloid
12.	345 g	530 g	+21.8 %	26.2 mg/100 g	70.5 %	2.0 %
14.	490 »	480 »	— 2.2 »	22.0 »	75.0 »	5.5 »
16.	425 »	430 »	+ 1.2 »	22.6 »	75.0 »	10.0 »
18.	475 »	500 »	+ 5.2 »	21.8 »	76.0 »	8.0 »
20.	455 »	520 »	+14.5 »	23.7 »	73.0 »	6.0 »
Mittelwerte	456 g	492 g	+ 8.1 %	23.2 ± 0.9	73.9 ± 0.9	6.3 ± 1.3

TAFEL 5

MEERSCHWEINCHEN NACH 30-TÄGIGER ZUFUHR DER KONTROLLÖSUNG UND NACHFOLGENDER ZUSÄTZLICHER ZUFUHR VON 0.025 G MTU TÄGLICH PER OS

Versuchstier Nr.	Gewicht zu Beginn des Versuchs	Gewicht nach Beendigung des Versuchs	Gewichtsveränderung	Gewicht der Schilddrüse	Epithel	Kolloid
22.	520 g	510 g	— 1.9 %	12.9 mg/100 g	62.0 %	26.0 %
24.	540 »	570 »	+ 5.5 »	14.2 »	79.0 »	11.0 »
26.	640 »	660 »	+ 3.1 »	10.0 »	70.0 »	14.0 »
28.	630 »	590 »	— 6.4 »	10.5 »	68.5 »	16.5 »
Mittelwerte	582 g	582 g	— 0.3 %	11.9 ± 0.9	69.9 ± 3.5	16.9 ± 3.3

TAFEL 6

MEERSCHWEINCHEN NACH 30-TÄGIGER ZUFUHR VON TÄGLICH 0.2 MG/KG DIGILAN INTRAPERITONEAL UND NACHFOLGENDER ZUSÄTZLICHER VERABREICHUNG VON 0.025 G MTU TÄGLICH PER OS

Versuchstier Nr.	Gewicht zu Beginn des Versuchs	Gewicht nach Beendigung des Versuchs	Gewichtsveränderung	Gewicht der Schilddrüse	Epithel	Kolloid
21.	560 g	525 g	— 6.2 %	18.9 mg/100 g	71.0 %	15.0 %
23.	560 »	530 »	— 5.4 »	14.3 »	72.5 »	13.0 »
27.	540 »	590 »	+ 9.3 »	16.3 »	77.0 »	9.0 »
29.	560 »	570 »	+ 1.8 »	14.6 »	78.5 »	10.0 »
Mittelwerte	555 g	554 g	— 0.5 %	16.0 ± 1.1	74.8 ± 1.8	11.8 ± 1.4

Während der ersten Versuchsreihe gelangte eine bemerkenswerte Gewichtabnahme der Tiere zur Beobachtung. Diese Gewichtabnahme war in sämtlichen Versuchsgruppen von gleicher Grössenordnung. Möglicherweise war die verwendete MTU-Menge zu gross. Das durchschnittliche relative Gewicht der Schilddrüsen war in der MTU-Gruppe 14.7 ± 1.6 mg und in der MTU & Digilan-Gruppe 16.6 ± 3.2 mg/100 g. Der Unterschied ist statistisch belanglos. Die durchschnittliche prozentuale Epithelmenge betrug in der MTU-Gruppe $74.0 \pm 2.8\%$ und in der MTU & Digilan-Gruppe $76.0 \pm 2.0\%$. Die Kolloidmenge in den entsprechenden Gruppen betrug $15.8 \pm 2.9\%$ bzw. $11.6 \pm 1.9\%$. In jener Gruppe war die prozentuale Epithelmenge ein wenig kleiner, die Kolloidmenge ein wenig grösser als in dieser. Die Unterschiede bleiben jedoch ohne statistische Bedeutung.

In der zweiten Versuchsreihe, wo die Dosierung von MTU 0.05 g pro Tier und Tag betrug, war eine Gewichtzunahme der Tiere beider Gruppen zu verzeichnen. Die Schilddrüsen der Tiere beider Gruppen waren vergrössert; das durchschnittliche relative Gewicht der Drüsen in der MTU-Gruppe betrug 25.5 ± 1.9 mg/100 g, in der MTU & Digilan-Gruppe 23.2 ± 0.9 mg/100 g. Ein statistisch ausschlaggebender Unterschied der beiden Gruppen lag nicht vor. Die durchschnittliche prozentuale Epithelmenge betrug bei der MTU-Gruppe $70.8 \pm 1.5\%$ und bei der MTU & Digilan-Gruppe $73.9 \pm 0.9\%$ Die entsprechenden Werte des Kolloids betrugen $7.7 \pm 1.6\%$ und $6.3 \pm 1.3\%$. Die Werte sind nicht statistisch von Bedeutung, doch liegt auch hier ein etwas kleinerer Epithelprozent und ein etwas grösserer Kolloidprozent bei der mit reinem MTU behandelten Gruppe von Versuchstieren vor.

In der letzten Versuchsreihe, wo die MTU-Gaben am niedrigsten dosiert wurden, 0.025 pro Tier und Tag, verblieb das Körpergewicht der Meerschweinchen während des Versuchs im grossen unverändert. Das durchschnittliche Gewicht der Schilddrüse betrug in der MTU-Gruppe 11.9 mg/100 g und in der MTU & Digilan-Gruppe 16.0 mg/100 g. Der Unterschied an durchschnittlichem Schilddrüsengewicht der beiden Gruppen ist statistisch belanglos. Die prozentuale Zusammensetzung der Schilddrüse ergab folgende Ziffern: Epithelprozent der MTU-Gruppe $69.9 \pm 3.5\%$, der MTU & Digilan-Gruppe $74.8 \pm 1.8\%$. Kolloidprozent der MTU-Gruppe $16.9 \pm 3.3\%$, der MTU & Digilan-Gruppe $11.8 \pm 1.4\%$. Auch diese Werte können

nicht als ausschlaggebend angesehen werden, jedoch liegt hier ebenfalls ein etwas grösserer Epithelprozent und ein etwas kleinerer Kolloidprozent bei den mit MTU und Digilan gleichzeitig behandelten Versuchstieren vor.

Aus Obigem geht hervor, dass die durch Methylthiourazil an Meerschweinchen hervorgerufene Vergösserung der Schilddrüse mit Hyperplasie des Epithels und Verminderung des Kolloids nicht durch gleichzeitige Digilangaben verhindert werden kann.

BESPRECHUNG

Wie bekannt bewirkt Methylthiourazil eine Verminderung der Hormonausscheidung der Schilddrüse. Hierbei steigt die Sekretion des thyreotropen Hormons der Hypophyse an, welches zu einer Hyperplasie der Schilddrüse führt. Andererseits wird die Schilddrüse, wie früher erwähnt, durch Digitalis inaktiviert, was einer dem thyreotropen Hormon entgegengesetzten Wirkung des Digitalis zugeschrieben wird (Bomskov et al., Kuusisto). Aus diesen Gründen liegt es nahe anzunehmen, dass Digitalis der die Schilddrüse aktivierenden Wirkung des Methylthiourazils entgegenwirkt. Dieses konnte nicht nachgewiesen werden. In den vorliegenden Versuchen war das histologische Bild im grossen gleich bei Meerschweinchen, denen reines Methylthiourazil zugeführt worden war, und bei Tieren, die unter gleichzeitiger Behandlung mit Methylthiourazil und Digitalis gestanden waren. Es scheint somit als ob Digitalis nicht imstande wäre, die aktivierende Wirkung des thyreotropen Hormons auf die Schilddrüse aufzuheben, welche durch die verwendeten Dosierungen von Methylthiourazil hervorgerufen worden war.

Es ist von Interesse festzustellen, dass die natürlichen oestrogenen Hormone, die ihrer chemischen Struktur nach dem Digitalis verwandt sind, ebenfalls wie das Digitalis die Schilddrüse inaktivieren (1, 5). Auf die durch Methylthiourazil aktivierte Schilddrüse wirkt das natürliche Oestrogen nicht inaktivierend (7).

ZUSAMMENFASSUNG

Die Verfasser haben die Einwirkung von Methylthiourazil und von Methylthiourazil und Digitalis bei gleichzeitiger Zufuhr auf das histologische Bild der Schilddrüse untersucht. Sie haben nach dem Verfahren von Uotila und Kannas die prozentuale Zusammensetzung der Drüse aus Kolloid und Epithel gemessen, und hierbei festgestellt, dass Digitalis nicht die aktivierende Wirkung des Methylthiourazils auf die Schilddrüse aufzuheben vermag.

LITERATURVERZEICHNIS

1. Aron, M., und Benoit, J.: Compt. rend. Soc. de biol. 1932:*109:*923.
2. Bensley, R. R.: Am. J. Anat. 1916:*19:*37.
3. Bomskov, C., Kaulla, K. N., und Maurath, J.: Arch. f. exper. Path. u. Pharmakol. 1941:*198:*203.
4. Halonen, P. I., Kuusisto, A. N., und Koskelo, P.: Cardiologia 1952 im Druck.
5. Karp, L., und Kostkiewicz, B.: Klin. Wchnschr. 1934:*13:*489.
6. Kuusisto, A. N.: Ann. med. exper. et biol. Fenniae 1952: suppl. im Druck.
7. Segaloff, A.: Endocrinology 1944:*35:*134.
8. Uotila, U., und Kannas, O.: Acta Endocrinologica 1952:*11:*49.

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ANTICOAGULANTS IN FUNGI

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The best-known anticoagulant deriving from the vegetable kingdom is coumarin which exerts an effect by indirectly lowering the blood prothrombin content. Agents with a preventive effect on the coagulation itself have also been isolated from the vegetable kingdom. The soya bean trypsin inhibitor which has been ascertained to have an effect in vivo too is probably the most investigated of these (17, 18). Among plant extracts which have been found to prevent coagulation, at any rate *in vitro*, the literature mentions *Viscum album* and spinach (4). The latex of *Ficus officinalis* is also known to possess the same characteristic (13).

Our intention has been to ascertain whether fungi include species whose extracts have preventive effect on the coagulation of blood.[1]

TECHNIQUE

The fungi were ground fine in a mortar and extracted in saline solution to a 10 per cent suspension. The suspension was allowed to remain overnight at 37° C[2] after which it was centrifuged and

[1] The fungi at our disposal were collected in the vicinity of Helsinki (Regio Nylandia).

[2] The study demonstrated that length of extraction time had no effect on the results. The same results were obtained if the extracts were centrifuged immediately after the addition of sodium chloride as with 12 hour's extraction time. However, for the sake of uniformity the technique reported in the text was applied in carrying out the investigation.

the clear or opalescent supernatant was used for the examinations. The tests were performed by pipetting into a test tube 0.5 cc of the fungus extract to be examined, and an equal part of blood was added to the extract. The blood for examination had been taken in a paraffin-coated vessel. After shaking the tube was allowed to remain at room temperature for one hour, after which the result was read. The result was regarded as positive if the blood did not coagulate, slightly positive with partial coagulation and negative if the blood changed into a solid coagulum.

Extracts that had reacted positively were titrated and their potency was examined after heating, dialysis and an addition of $CaCl_2$. Between examinations the extracts were kept deep-frozen (—20° C). Their activity remained unchanged in the course of one year's storage.

RESULTS

The material comprised 175 species. The majority of them proved inactive (161 species) with this technique. The number of slightly positive species was 10 and more pronounced positivity was ascertained in four species. The group of slightly positive species included certain samples whose extracts reacted positively when undiluted though other samples of the same species were inactive. In species classified as positive every individual examined proved active. The negative species are enumerated in List 1, the slightly positive in List 2. The fungi are listed in alphabetical order of genus.

List 1

Negative Fungi

Hymenomycetes

Nomenclature principally according to Karstén (7, 8) supplemented with other authors listed in the bibliography (1, 5, 6, 9, 10, 11, 14, 15, 16).

Amanita mappa (Batsch) Fr.
» muscaria (L) Fr.
» muscaria forma aureola (Kalchbr.)
» porphyria (Alb. & Schw.) Fr.
» rubescens (Pers.) Fr.
Amanitopsis vaginata (Bull.) Roz.
Armillaria mellea (Vahl.) Fr.
Bjerkandera irregularis (Scop.) Karst. = Leptoporus amorphus (Fr.) Quél.

Boletus bovinus Fr.
» edulis Bull.
» elegans Schum.
» felleus (Bull.) Fr.
» luteus L.
» piperatus Bull.
» scaber Bull.
» scaber var. niveus Fr. = B. holopus Rostk.
» subtomentosus L.
» variegatus Swartz
» versipellis Fr.
Calocera viscosa (Pers.) Fr.
Calodon cyathiformis (Schaeff.) Quél.
Cantharellus auranthiacus (Wulf.) Fr.
» cibarius Fr.
» infundibuliformis (Scop.) Fr.
» umbonatus (Pers.) Fr. = C. muscoides (Wulf.) Karst.
Clavaria flava Schaeff.
» ligula Schaeff.
Clitocybe aggregata (Schaeff.) var. sphaerospora Lange
» angustissima (Lasch) Fr.
» clavipes (Pers.) Fr.
» connata (Schum.) Fr.
» diatreta Fr.
» dicolor (Pers.) Fr.
» gilva (Pers.) Fr.
» infundibuliformis (Schaeff.) Fr.
» nebularis (Batsch) Fr.
» obsoleta (Batsch) Fr.
» odora (Bull.) Fr.
» rivulosa (Pers.) Fr.
Collybia butyracea (Bull.) Fr.
» confluens (Pers.) Fr.
» dryophila (Bull.) Fr.
» maculata (Alb. & Schw.) Fr.
» velutipes (Curt.) Fr.
Coprinus atramentarius (Bull.) Fr.
» comatus (Schum.) Fr.
» micaceus (Bull.) Fr.
Cortinarius alboviolaceus Fr.
» anomalus Fr.
» armillatus Fr.
» bolaris (Pers.) Fr.
» camphoratus Fr.
» cinnamomeus (L.) Fr.
» collinitus (Pers.) Fr.
» elatior Fr.

Cortinarius flexipes Fr.
» fulgens (Alb. & Schw.) Fr. sensu Cooke (Lange 1938)
» gentilis Fr.
» obtusus Fr.
» pholideus Fr.
» sanguineus (Wulf) Fr.
» semisanguineus Fr.
» traganus Fr.
Flammula alnicola Fr.
» flavida (Schaeff.) Fr.
Fomitopsis pinicola (Swartz) Karst. = Ungulina marginata (Fr.) Pat.
Galera tenera (Schaeff.) Fr.
Gomphidius glutinosus (Schaeff.) Fr.
» roseus Fr.
» viscidus (L.) Fr.
Hansenia hirsuta (Wulf.) Karst. = Coriolus hirsutus (Wulf.) Quél.
» zonata (Fr.) Karst. = Coriolus zonatus (Fr.) Quél.
Hebeloma crustuliniforme (Bull.) Fr.
Hydnum corrugatum Fr.
» repandum L. = Tyrodon repandus (L.) Karst.
» rufescens Pers. = Tyrodon rufescens (Pers.) Karst.
Hygrophorus agathosmus Fr.
» eburneus (Bull.) Fr.
» hypothejus Fr.
» olivaceoalbus Fr.
Hypholoma candolleanum Fr.
Inocybe asterospora Quél.
» geophylla (Sow.) Fr. var. alba Lange
Inonotus radiatus (Sow.) Karst. = Xanthocrous radiatus (Sow.) Pat.
Laccaria laccata (Scop.) Cook. var. amethystina Bolt.
» laccata var. proxima Boud.
Lactarius camphoratus (Bull.) Karst.
» deliciosus (L.) Fr.
» glyciosmus Fr. sensu Knauth & Neuhoff = L. confuses Lundell
» helvus Fr.
» rufus (Scop.) Fr.
» subdulcis (Pers.) Fr. sensu Karst. Lundell etc. = L. thejogalus (Bull.) Fr. s. Knauth & Neuhoff.
» torminosus (Schaeff.) Fr.
» trivialis Fr.
» turpis (Weinm.) Fr.
» vietus Fr.
Lenzites betulina (L.) Fr.
Lenzitina saepiaria (Schaeff.) Fr. Karst.
Lepiota amianthina (Scop.) Fr.
» carcharias (Pers.) Fr.
» clypeolaria (Bull.) Fr.

Lepiota cristata (Alb. & Schw.) Fr.
» granulosa (Batsch) Fr.
Marasmius oreades (Bolt.) Fr.
» peronatus (Bolt.) Fr.
» scorodonius Fr.
Mycena alcalina Fr.
» epipterygia (Scop.) Fr.
» galericulata (Scop.) Fr.
» metata Fr.
» pura (Pers.) Fr.
» rosella Fr.
» vulgaris (Pers.) Fr.
Naematoloma capnoides (Fr.) Karst.
» sublateritium (Schaeff.) Karst.
Naucoria escharoides Fr.
» scolecina Fr.
Nolanea hirtipes (Schum.) Fr.
Panaeolus campanulatus (L.) Fr.
Paxillus atromentosus (Batsch) Fr.
» involutus (Batsch) Fr.
Pholiota mutabilis (Schaeff.) Fr.
» squarrosa (O. F. Müll.) Fr.
Pleurotus mitis (Pers.) Fr.
Polyporus ovinus (Schaeff.) Fr.
Polystictus Schweinitzii Fr. = Phaeolus Schweinitzii (Fr.) Pat.
Psalliota arvensis (Schaeff.) Fr.
» hortensis Cooke coll.
Psathyra fusca (Schum.) Lange
Psathyrella disseminata (Pers.) Fr.
Psilocybe spadicea Fr.
» uda (Pers.) Fr.
Russula claroflava Grove
» decolorans Fr.
» emetica (Schaeff.) Fr.
» fragilis (Pers.) Fr. = R. Mairei Singer (Lange 1940)
» paludosa Britz = R. elatior Lindbl.
» puellaris Fr.
» vesca Fr.
Sarcodon imbricatus (L.) Quél.
Sparassis crispa (Wulf.) Fr.
Stropharia depilata (Pers.) Fr.
» semiglobata (Batsch) Fr.
Trametes cinnabarina (Jacq.) Fr.
Tricholoma albobrunneum (Pers.) Fr. = T. striatum (Schaeff.) Quél.
» album (Schaeff.) Fr.
» flavobrunneum Fr.
» grammopodium (Bull.) Fr.

Tricholoma imbricatum Fr.
» pessundatum Fr.
» rutilans (Schaeff.) Fr.
» vaccinum (Pers.) Fr.
» virgatum Fr.
Tubaria furfuracea (Pers.) W. Sm.

Gasteromycetes
Nomenclature according to Th. Fries (3)
Bovista nigrescens Pers.
Lycoperdon pyriforme Pers.

Discomycetes
Nomenclature according to W. Migula (12)
Otidea leporina (Batsch) Fuck.
» onotica (Pers.) Fuck.
Peziza aurantia Müller = Aleuria aurantiaca Fuck.
Rhytisma acerinum (Pers.) Fr.

Myxomycetes
Nomenclature according to Rob. Fries (2)
Lycogala Epidendrum (L.) Fr.

List 2.

Slightly Positive Fungi

Hymenomycetes (Nomenclature as in list 1.)

Clitocybe ditopus Fr.
Clitopilus orcella (Bull.) Fr.
» prunulus (Scop.) Fr.
Cortinarius brunnueus (Pers.) Fr.
Pleurotus ulmarius (Bull.) Fr.
Stropharia aeruginosa (Curt.) Fr.
» albocyanea (Desm.) Fr.
Tricholoma nudum (Bull.) F.
» saponaceum Fr.

Gasteromycetes (Nomenclature as in List 1.)
Lycoperdon perlatum Pers. = L. gemmatum Fr.

POSITIVE SPECIES

4 species proved to be positive: *Collybia platyphylla* (Pers.) Fr., *Pluteus cervinus* (Schaeff.) Fr., *Polyporus confluens* (Schaeff.) Fr. and *Tricholoma equestse* (L.) Fr.

THE EFFECT OF HEAT ON ACTIVE EXTRACTS

Tubes containing the extracts were kept for half an hour in water of a certain temperature and a comparison was made between their coagulating ability before and after heating. The results are presented in Table 1.

TABLE 1

THE EFFECT OF $^1/_2$-HOUR'S HEATING ON ACTIVE MUSHROOM EXTRACTS

		Collybia platyphylla						*Pluteus cervinus*						*Polyporus confluens*						*Tricholoma equestre*					
		$^1/_1$	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$	$^1/_{32}$	$^1/_1$	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$	$^1/_{32}$	$^1/_1$	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$	$^1/_{32}$	$^1/_1$	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$	$^1/_{32}$
Before heating		+	+	+	—	—	—	+	+	+	±	—	—	+	+	+	+	—	—	+	+	+	+	+	±
After heating for ½ h. in	56°C	+	+	+	—	—	—	+	+	+	—	—	—	+	+	+	+	—	—	+	+	—	—	—	—
	60°C	+	+	+	—	—	—			—				+	+	+	±	—	—			—			
	63°C			—				+	+	+	—	—	—			—				—	—	—	—	—	—
	66°C			—				+	+	±	—	—	—			—				—	—	—	—	—	—
	70°C	+	+	+	—	—	—	+	+	±	—	—	—	±	—	—	—	—	—	—	—	—	—	—	—
	100°C	+	+	+	—	—	—	+	+	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Explanation of sympols used:
+ the blood does not coagulate
± the blood coagulates partly
— the blood coagulates

Factors present in *Polyporus confluens* and *Tricholoma equestre* are destroyed when the extracts are heated for half an hour at 56—70° C; in *Collybia platyphylla* and *Pluteus cervinus* the factors are thermostabile and stand $^1/_2$-hour's heating at 100° C without weakening.

THE REACTION OF THE EXTRACTS TO $CACL_2$ ADDITION

A $CaCl_2$ solution of approximately 240 mg Ca per 100 cc of saline was prepared. The anticoagulating ability of the mushroom extracts was examined before and after addition of the same volume of $CaCl_2$ solution to each dilution. As controls were used 1.85 per cent potassium oxalate and 3.8 per cent sodium citrate solutions. The results are presented in Table 2.

All these fungus extracts possess the same property: there is no diminution in their anticoagulating ability after addition of

TABLE 2

THE ANTICOAGULATING ABILITY BEFORE AND AFTER ADDITION OF $CACL_2$ SOLUTION

	Before addition of $CaCl_2$								After addition of $CaCl_2$							
	$^1/_1$	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$	$^1/_{32}$	$^1/_{64}$	$^1/_{128}$	$^1/_1$	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$	$^1/_{32}$	$^1/_{64}$	$^1/_{128}$
Sodium citrate	+	+	+	+	+	—	—	—	±	—	—	—	—	—	—	—
Potassium oxalate ..	+	+	+	+	±	—	—	—	—	—	—	—	—	—	—	—
Collybia platyphylla	+	+	+	+	+	—	—	—	+	+	+	±	—	—	—	—
Pluteus cervinus	+	+	+	+	+	±	—	—	+	+	+	+	+	—	—	—
Polyporus confluens	+	+	+	+	±	—	—	—	+	+	+	±	—	—	—	—
Tricholoma equestre	+	+	+	+	+	+	—	—	+	+	+	+	±	—	—	—

$CaCl_2$ whereas the sodium citrate and potassium oxalate solutions used as controls lost their ability almost completely.

On the addition of calcium chloride an obvious precipitate formed in the tubes containing potassium oxalate and Collybia and Pluteus extracts.

DIALYZATION

The dialyzability of the anticoagulant factors of mushrooms was examined by dialyzing the extracts for 48 hours at room temperature through a cellulose membrane against saline. All the factors were found to lose some of their activity during the process, *Polyporus confluens* and *Tricholoma equestre* to a smaller extent, *Collybia platyphylla* and *Pluteus cervinus* most, but not much.

SUMMARY

The anticoagulant property of saline extracts prepared from fungi (1: 10) has been investigated. Of the 175 species examined 161 were completely inactive when studied by this method. Slight activity was ascertained in 10 species and stronger activity in 4 species. Of these the factors present in *Tricholoma equestre* and *Polyporus confluens* proved to be thermolabile and were destroyed at 56—70° C; the factors in *Pluteus cervinus* and *Collybia platyphylla* were thermostabile and withstood $^1/_2$-hour's heating at 100° C. Addition of $CaCl_2$ did not affect their anticoagulating ability. 48-hour dialysis diminished slightly the titer of the extracts.

Acknowledgement. — This investigation was aided by a grant from the Jenny and Antti Wihuri's Foundation.

REFERENCES

1. Bourdot, H., and Galzin, A.: Hyménomycetes de France I, Marcel Bry, Sceaux 1927.
2. Fries, Rob. E.: Sv. Botanisk Tidskrift, Sth. 1912:*6:*721.
3. Fries, Thore C. E.: Arkiv för Botanik, K. Sv. Vetenskapsakademien, Sth, 1921:*17:*9:1.
4. Havas, L. J.: Experimentia 1948:*4:*69.
5. Ingelström, Einar: Svampflora, Nordisk Rotogravyr, Sth. 1940.
6. Kallenbach, F.: Die Röhrlinge (Boletaceae), Die Pilze Mitteleuropas I Werner Klinkhardt, Leipzig 1926—42.
7. Karsten, P. A.: Rysslands, Finlands och den Skandinaviska Halvöns hattsvampar I—II, Bidr. t. känned. Finl. nat. o. folk, 32 o. 37, Finska Vetenskaps Societeten, Helsingfors 1879 o. 1882.
8. Karsten, P. A.: Kritisk öfversigt af Finlands Basidsvampar, Ibid. 48, 1889.
9. Knauth, B., and Neuhoff, W.: Die Milchlinge (Lactarii), Die Pilze Mitteleuropas II, Werner Klinkhardt, Leipzig 1935—43.
10. Lange, Jakob E.: Flora Agaricina Danica I—V, Soc. Advanc. of Mycology and Danish Bot. Soc., Copenhagen 1935—40.
11. Lundell, Seth, and Nannfeldt, J. A.: Fungi Exsiccati Suecici, praesertim Upsalienses Fasc. XV—XVI, Inst. Syst. Bot., Uppsala 1939.
12. Migula, W.: Kryptogamen-Flora von Deutschland, Deutsch-Österreich und der Schweiz, III, 3 Teil, 2. Abt., Friedrich von Zezschwitz, Gera, R. 1913.
13. de Paula, H.: Rev. Brasil. Med. oct. 1949:*6:*669.
14. Pearson, A. A.: British Boleti, A. Brown & Sons, Limited, London and Hull 1950.
15. Pearson, A. A.: The Genus Lactarius, A. Brown & Sons, Limited, London ond Hull 1950.
16. Pearson, A. A.: The Genus Russula, A. Brown & Sons, Limited, London and Hull 1950.
17. Tagnon, and Soulier: Proc. Soc. Exper. Biol. et Med. 1946:*61:*440.
18. Tagnon, and Soulier: Blood oct. 1948:*3:*1161.

FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

THE EFFECT OF AUREOMYCIN ON TADPOLES FED ON LIVER POWDER

PRELIMINARY REPORT

by

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(Received for publication October 8, 1952)

Aureomycin added to food has been ascertained to have a growth-promoting effect in several warm-blooded animals. It has been found to stimulate growth in chicken (4, 9), turkey (8), pig (1, 5), calf (7) and rat (2). This effect has been observed to be most pronounced if the food contains vitamin B_{12}.

The writers' own investigation aims at discovering whether aureomycin affects the growth of tadpoles fed on liver powder.

MATERIAL AND RESULTS

The material consisted of 120 tadpoles (*Rana temporaria*) which were collected from the same small pool on July 6, 1952. They were of the same size and had no macroscopically discernible hindlegs. The material was subdivided into three groups of 40 animals, each placed in a separate 10 litre aquarium. As food each group was given daily an abundant and equal portion of powder ground from dried liver. The water was changed every fifth day. The first group served as control material, the second group was given 50 mg of aureomycin (Lederle) mixed with the liver powder at the beginning of the experiment, the same dose being repeated after five and ten days; the third group, in the same way, was given 150 mg of aureomycin at the beginning of the test and after five

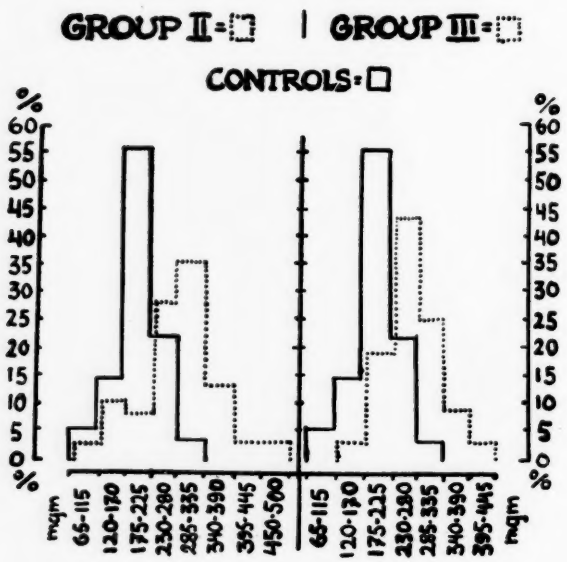

Fig. 1. — Weight distribution of tadpoles.

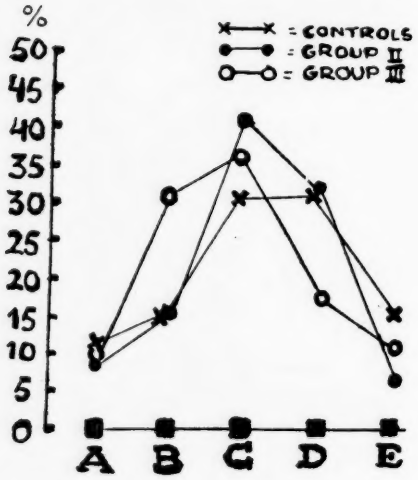

Fig. 2. — Distribution of leg lengths.

A = no macroscopically discernible legs
B = small hindlegs
C = intermediate hindlegs
D = great hindlegs
E = forelegs and hindlegs

and ten days. After the fifteenth day the tadpoles were killed, weighed and their stage of metamorphosis ascertained from their legs.

The results are presented in Figs. 1 and 2. The mean weight of the control group was 203.3 ± 7.9 mg (σ 47.6), that of the second group 276.9 ± 12.6 mg (σ 79.8) and of the third group 268.1 ± 9.4 mg (σ 57.7). The difference between the control group and both aureomycin groups was statistically significant; no difference could be registered between the mean weights of the aureomycin groups. Fig. 2 illustrating the metamorphosis seems to take the same course for all the groups, but as the stage of metamorphosis was fairly young at the end of the experiment it may not be possible to draw exact conclusions from it.

DISCUSSION

Recent investigations have proved that protein supplements derived from the animal kingdom have a growth-promoting effect in several animals that have been fed solely on a vegetable diet. This so-called animal protein factor has been regarded as identical with vitamin B_{12}, or has been considered at least to contain vitamin B_{12} (11, 12). Aureomycin has also been proved to have such an animal protein factor effect, explained by aureomycin's eliminating effect on toxic or vitamin-consuming intestinal micro-organisms favouring vitamin B_{12} producing micro-organisms (11). Wright and his colleagues (10) state that vitamin B_{12} for its part may act as a coenzyme in the thymine-thymidine-thymonucleic acid metabolism, which would explain its growth-promoting effect.

On the basis of the writers' results it does seem that the growth-promoting effect of aureomycin established in warm-blooded animals is also present when tadpoles are used as the test animals. The tadpole and its metamorphosis, regulated by the thyroid, seems in the writers' opinion to be a suitable examination object for investigations of growth as the metamorphosis offers a kind of fixed point for the correlation of exogenous growth-factors and endogenous growth-regulators.

SUMMARY

The object of the investigation was to ascertain whether aureomycin, which promotes growth in several warm-blooded animals when added to food, has a growth-promoting effect also on tadpoles fed on liver. Of three groups of 40 tadpoles one served as a control series and two were given aureomycin mixed with food. The mean weight of the control series was 203 mg at the termination of the experiment, the corresponding figures for the series given aureomycin being 276 and 268 mg. The differences between the control series and the aureomycin series were statistically significant. No definite differences could be observed in the stage of metamorphosis reached by the end of the experiment.

REFERENCES

1. Catron, D. V., Maddock, H. M., Speer, V. C., and Vohs, R. L.: Antibiotics & Chemotherapy (Wash.) 1951:*1:*31.
2. Cravioto-Munoz, J., Poncher, H. G., and Waisman, H. A.: Proc. Soc. Exp. Biol. Med. 1951:*77:*18.
3. Edwards, H. M., Cunha, T. J., Meadows, G. B., Sewell, R. F., and Shawver, C. B.: Ibidem 1950:*75:*445.
4. Harned, B. K., Cunningham, R. W., Clark, M. C., Cosgrove, R., Hine, C. H., McCanley, W. J., Stokey, E., Vessey, R. E., Yuda, N. N., and Subba Row, Y.: Ann. N. Y. Acad. Sci. 1948:*51:*182.
5. Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. B.: Arch. Biochem. 1950: *26:*326.
6. Oleson, J. J., Hutchings, B. L., and Whitehill, A. R.: Ibidem 1950:*29:*334.
7. Rusoff, L. L., Davis, A. V., and Alford, J. A.: J. Nutrition 1951: *45:*289.
8. Stokstad, E. L. R., and Jukes, T. H.: Poultry Science 1950:*29:*611.
9. Stokstad, E. L. R., and Jukes, T. H.: Proc. Soc. Exp. Biol. Med. 1951:*76:*73.
10. Wright, L. D., Skeggs, H. R., and Huff, J. W.: J. Biol. Chem. 1948: *175:*475.
11. Zink, A.: Internat. Rev. of Vitamin-Research 1952:*23:*471.
12. Zucker, T. F., and Zucker, L. M.: Vitamins and Hormones 1950:*8:*2.

FROM THE DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF HELSINKI

BROMSULPHALEIN EXCRETION IN DOGS DURING ACUTE HYPOXIA

by

EEVA JALAVISTO and H. LYBECK

(Received for publication October 12, 1952.)

The effects of anoxia on the functions of the liver are still poorly understood. Whereas it is a well substantiated fact that ischemic conditions cause definite impairment of hepatic function (9, 14) the effect of arterial (anoxic) hypoxia has received relatively little attention. However, histological signs of degeneration in the central portion of the hepatic lobules have been recorded after exposure to extreme low atmospheric oxygen tensions (5, 13, 19) and Rich (17) mentions a decrease of bilirubin excretion both after low oxygen pressure breathing and in experimental haemorrhagic anaemia. Otherwise the effect of anoxic hypoxia upon the many different functions of the liver have remained, as far as we know, practically unanalyzed.

Cells with secretory function are usually dependent upon adequate oxygen supply. So e.g. gastric acid secretion (4, 7, 10) and pancreatic secretion (6) are depressed in low atmospheric pressure. Since hepatic tissue has an excretory function, it is reasonable to ask whether this function is as well sensitive to lack of oxygen as experiments of Rich with bilirubin seem to indicate.

Of the liver function tests, the bromsulphalein excretion test is considered as one of the most sensitive (18). The mechanism of removal of bromsulphalein (BS), however, seems not to be only a function of the parenchymal hepatic cells (8); the first rapid decrease in blood bromsulphalein content after injection of the

dye dependens obviously upon the action of the reticulo-endothelial (RES) Kupfer cells. A previous blocking of the RES results namely in a slight retention of the dye (2, 3, 11, 15, 16). The two different mechanisms are working with different speeds so that it may be possible to separate these two modes of removal as two different phases in the curves of elimination. According to Cantarow, Wirts, Snape and Miller most of the BS is eliminated from the blood within 15—45 minutes, the excretion into the bile, however going on for hours. If, as pointed out by Lavers and co-workers (12) the excretion into the bile by the parenchymal hepatic cells is deficient, the RES cells become blocked with the dyestuff and cease to remove the dye as effectively as before. In that way both mechanisms may appear deficient in hepatic damage — probably an explanation of the fact that of several clinical function tests the BS-excretion test seems to be one of the best.

The aim of our study was to see whether moderately severe hypoxia induced by breathing of a low oxygen-nitrogen gas mixture would affect the removal of BS from the blood in dogs.

METHODS

The experiments were performed on unanesthetized mongrel dogs. The oxygen-nitrogen gas mixture containing 6—8% oxygen was administered through a gas mask, specially constructed for use on dogs.[1] The dogs were all bread up in our laboratory and had become used to experimental work, and did not, as a rule, object to wearing the mask.

After some preliminary experiments carried out in the usual way the bromsulphalein test was modified to some extent. In order to increase its sensitivity the BS was injected twice with only 5 or 10 minutes interposed between the two injections of 5 mg BS/kg body weight. It was thought that the RES-cells would be blocked by the first injection and the excretory function of hepatic cells would perhaps play a relatively more prominent role in the elimination of the dye under these circumstances than it would after only one single injection. Blood samples were drawn with 2—5 minutes intervals during 40—45 minutes from a vein not used for injection of the dye. The blood samples were analyzed with the usual bloch comparator method, which yields more reliable results than the photometric methods if the sera are not quite clear. A small comparator was used requiring only ca 2 ml. serum for comparison. As usually the samples were made alkaline with one drop of 10% sodium hydroxide

[1] The masks were supplied by the Finnish Defence Forces to which we wish to express our sincere thanks.

solution and compared with an acidified sample (one drop of 5% hydrochloric acid). The result was given in units which correspond to the »percentage» values in the routine BS-test i.e. 4 mg/100 ml = 100 per cent = 100 units. With every dog a control determination under normal conditions and an experiment with breathing of 6 or 8% oxygen were performed on different days. The administration of the low oxygen mixture was started 10—14 minutes before the first injection of the dye, the dog standing in a Pavlov stand. The mixture came from a container with a breathing valve of the type used in oxygen inhalators. The duration of the experiment was from beginning of administration of the low oxygen mixture 50—60 minutes in all.

RESULTS

In the preliminary experiments with the dogs »Diogeneia», »Pelle» and »Sokrates» practically all BS was removed from the blood in 20—25 minutes both in control experiments and in severe anoxia (6—7% O_2). The results of the double injection technique experiments were extremely uniform possible with exception of one dog, »Socrates». In all instances the early phases of the control and hypoxic curves of all dogs overlap. The elimination proceeds with extreme rapidity. In 5 min. the concentration of the dye fell to some 25—45 units. When the second injection 7 minutes after the first was administered, the fall in dye concentration was again practically as rapid as after the first injection. 20 minutes after the first injection (13 minutes after the second) there were usually only 25 units of the dye left. Thereafter the slope of the curves

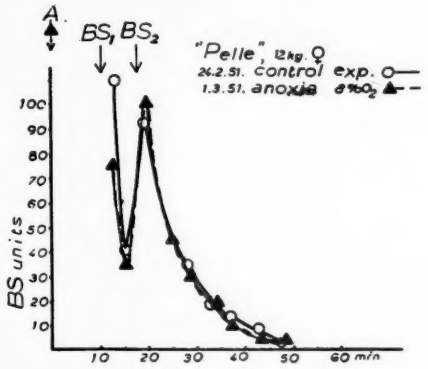

Fig. 1. — Blood bromsulphalein content after double intravenous injections. At BS_1 and BS_2 i.v. injections of 5 mg/kg body weight bromsulphalein. At A breathing of 8 per cent oxygen started.

decreased. After 45 minutes no dye or only traces of it were left. This description holds true as well for the control as for the anoxia experiments as may be seen from figures 1—4. In fig. 5 a slightly different picture is seen. With the dog »Socrates» three experiments were performed: one control, one with breathing of 100 per cent oxygen through the gas mask and one with 8 per cent oxygen-nitrogen mixture. When the »anoxic» and »control» curves are compared a slight retention of the dye seems to be present in the hypoxic experiment. When on the other hand the experiment with breathing of pure oxygen is considered the retention of the

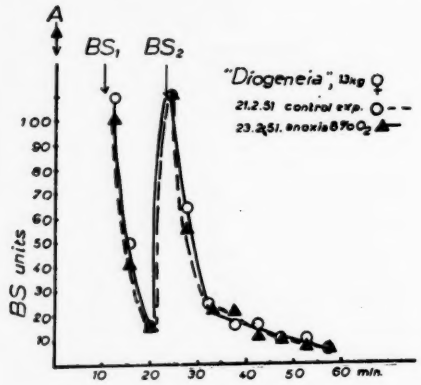

Fig. 2. — Blood bromsulphalein content after double BS injections. At BS_1 and BS_2 i.v. injections of 5 mg/kg body weight bromsulphalein. At A breathing of 8 per cent oxygen started.

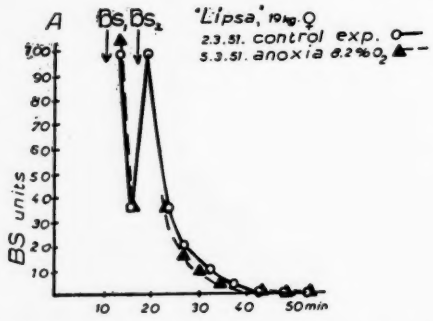

Fig. 3. — Blood bromsulphalein content after double BS injections. At BS_1 and BS_2 i.v. injections of 5 mg/kg body weight bromsulphalein. At A breathing of 8 per cent oxygen started.

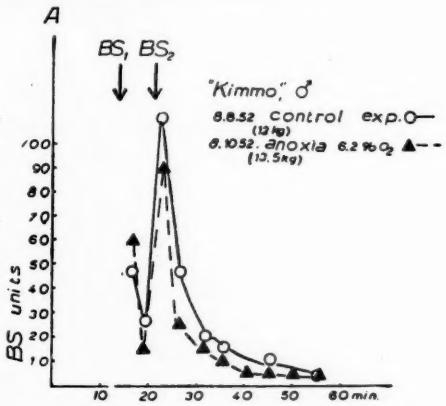

Fig. 4. — Blood bromsulphalein content after double BS injections. At BS_1 and BS_2 i.v. injections of 5 mg/kg body weight bromsulphalein. At A breathing of 6.2 per cent oxygen started.

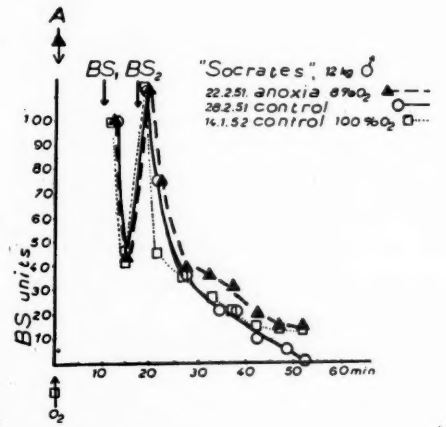

Fig. 5. — Blood bromsulphalein content after double BS injections. At BS_1 and BS_2 i.v. injections of 5 mg/kg body weight bromsulphalein. At A O_2, breathing of oxygen 8 and 100 per cent, respectively, started.

dye after 42 minutes is as great as in the anoxic experiment, whereas the earlier phases of the excretion curve correspond to the control curve. The differences therefore represent more likely random variations in the excretory function than an effect of hypoxia on it.

Because of the fair consistency of the results no further experiments were performed.

DISCUSSION

Obviously the administration during 50 min. of 6—8 per cent oxygen mixture does not affect the excretion of bromsulphalein. This concentration corresponds to a simulated altitude of 6750—8400 meters and is enough conspicuously to depress the response of pancreatic secretion to continous infusion of secretin (6). It remains unsettled whether a further reduction in oxygen content of the air would produce some effect. Anyhow, the experiments indicate with certainty that the elimination of BS from the blood is not as sensitive against lack of oxygen as e.g. the function of pancreatic cells. The following questions therefore arise. 1) Is the process of elimination of bromsulphalein at all dependent upon the supply of oxygen, or is it an essentially anaerobic process 2) is the circulatory compensation of the arterial oxygen deficit as efficient as to spare the hepatic cells from lack of oxygen under the experimental conditions of this study.

These questions cannot be answered in experiments with unanesthetized dogs. A more severe anoxia leads mostly to vomitus or to unconsciousness and cramps which make the use of a gas mask very difficult. The determination of the hepatic blood flow and oxygen consumption on the other hand, is under these experimental conditions hardly possible (the excretion of BS being under investigation and at the same time basis of the only feasible »clearance» method for calculation of blood flow!)

In vitro experiments of BS removal from blood plasma by the liver of the rat have led to inconsistent results. According to Brauer and Pesotti (1) the binding of BS by the liver cells is not an enzymatic process, the cyanides, mercury and fluorides being incapable to inhibit the removal of BS from plasma by liver slices. In vitro and in vivo experiments are, however, not always consistent. E.g. carbontetrachloride which is inefficient in vitro, does prevent the removal of BS in vivo. It is probable that the *in vitro* experiments represent only the first rapid phase of BS removal by the RES and not at all the excretory functions of the hepatic parenchymal cells. A possible explanation of our experiments would analogously be that in spite of the double injections the elimination curve is mainly determined by the functioning of the RES and is consequently not affected by lack of oxygen, however severe.

At the time being, it seems more appropriate to extend the studies to other hepatic functions under anoxia, and try to elucidate the possible circulatory compensatory mechanisms in more direct experiments with anesthetized animals. Experiments on these lines are going on at our laboratory.

SUMMARY

1. Bromsulphalein excretion tests have been performed with 5 dogs during severe and moderately severe anoxia, i.e. the dogs breathing 6—8 per cent oxygen-nitrogen mixture.

2. The test was modified to a double 5 mg/kg body weight injection of BS and the BS content was followed during 45 min. by withdrawing blood samples at 2—5 min. intervals. The results are seen in figures 1—5.

3. The hypoxia does not as a rule impair the elimination of BS by the liver.

REFERENCES

1. Brauer, W., and Pesotti, R. L.: Quot. from Ber. physiol. 1951: *143:*284.
2. Cantarow, A., and Wirts, C. W. J.: Ann. J. Digest. Dis. 1943:*10:*261.
3. Cantarow, A., Wirts, C. W. J., Snape, W. J., and Miller, L. L.: Am. J. Physiol. 1948:*154:*211.
4. Delrue, G.: Arch. Int. Physiol. 1934:*38:*126.
5. Florentin, P., Grandpierre, R., Grognot, P., and Royer, J.: C. r. Soc. Biol. 1944:*138:*280.
6. Hartiala, K. J. W.: Effect of Anoxic Anoxia on the Humorally Stimulated Pancreatic Secretion. — Ann. Acad. Sci. Fenn. A. V., F. 25. 1951.
7. Hartiala, K. J. W., and Karvonen, M.: Acta Physiol. Scand. 1946: *11:*85.
8. Herlitz, C.: Acta paediatr. 1931:*12:*Suppl. 5, 1.
9. Himsworth, H. P.: The Liver and Its Diseases. — Oxford, Blackwell, 1950.
10. Karvinen, E., and Karvonen, M. J.: Ann. Med. Exp. and Biol. Fenn. 1949:*27:*59.
11. Klein, R. I., and Levinson, S. A.: Proc. Soc. Exp. Biol. Med. 1933: *31:*179.
12. Lavers, G. D., Cole, W. H., Keeton, R. W., Gephardt, M. C., and Dysciewicz, J. M.: J. Lab. and Clin. Med. 1949:*34:*965.
13. Martin, G. H., Bunting, C. H., and Lowenhart, A. S.: Quot. by Himsworth.

14. McMichael, J.: Quart. J. Exp. Physiol. 1937:*27:*36.
15. Mills, M. A., and Dragstedt, C. A.: Proc. Exp. Biol. and Med. 1936:*34:*228.
16. Mills, M. A., and Dragstedt, C. A.: Arch. Int. Med. 1938:*62:*216.
17. Rich, A. R.: Bull. J. Hopkins Hosp. 1930:*47:*338.
18. Ricketts, W. E., Kirsner, J. B., Palmer, W. L., and Sterling, N.: J. Lab. Clin. Med. 1950:*35:*403.
19. Rosin, A.: Zieglers Beiträge 1928:*80:*622.

FROM THE DEPARTMENT OF FORENSIC MEDICINE, UNIVERSITY OF HELSINKI

EXPERIMENTAL ALTERATIONS OF CELL SIZE AND MITOTIC ACTIVITY IN THE OUTER ORBITAL GLAND OF THE WHITE RAT

VII

INFLUENCE OF FRACTIONED ROENTGEN IRRADIATION

by

H. TEIR and K. PYÖRÄLÄ

(Received for publication October 17, 1952)

Roentgen irradiation of the outer orbital gland of 2-week-old rats with single doses of 600, 1000, 1500 and 3000 r influences the normal development of the nuclear classes in such a way that the higher of the four nuclear classes normally occurring in the organ develop earlier than usual while, moreover, higher nuclear classes than normal occur in the organ (11). With single doses of 100, 200 and 400 r, no noticeable changes in the relation between the nuclear classes were obtained.

The purpose of this investigation was to find out whether the same effect can be obtained with repeated small roentgen doses as with a bigger single dose. For this purpose the right outer orbital gland was irradiated at the age of 12 days for 10 consecutive days with either 200 or 400 r. Otherwise the experimental conditions and the methods of investigation were the same as before (11).

ROENTGEN IRRADIATION WITH 200 R FOR 10 CONSECUTIVE DAYS

The seven experimental animals were divided into five age groups: two animals were killed at the age of 1 month, one at 2 and one at 4 months, and two at the age of 5 months and 3 weeks.

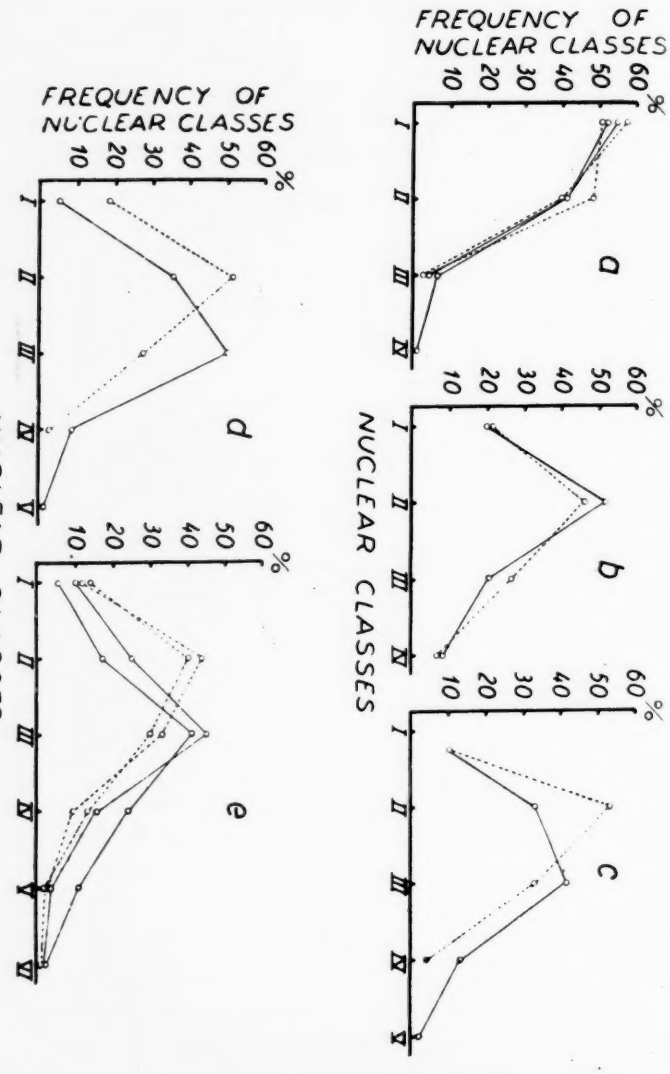

Fig. 1. — Diagram showing the nuclear class ratio in the outer orbital glands of rats irradiated with 200 r × 10; a = at the age of 1 month, b = 2 months, c = 4 months, d = 5 months and 3 weeks, e = 6 months and 3 weeks. The solid line indicates the irradiated right outer orbital gland and the broken line the left gland.

In the two first groups no difference between the nuclear class curve of the two outer orbital glands could be observed (fig. 1). In the three latter, however, a clear deviation to the right of the nuclear class curve could be noticed in the treated right gland. This revealed itself, for instance, in the dominance of the third nuclear class (K_4 and 2 K_2) whereas the second nuclear class (K_2 and 2 K_1) was dominant in the left gland. In the two oldest animals

a slight deviation to the right was also noticed in the left gland, and both the fifth and the sixth nuclear classes were also represented in it.

In one of the youngest rats the mitotic ratio was considerably higher in the irradiated gland than in the left one (Table 1). The number of mitoses in the adult animals was a little higher than normal since mitoses are but exceptionally found in adult animals (8).

TABLE 1

MITOTIC RATIO IN THE OUTER ORBITAL GLAND AFTER PROLONGED ROENTGEN IRRADIATION

Experiment	Rats		Time After the Last Irradiation, Months	Mitotic Ratio in the Outer Orbital Glands		Comments
	No.	Age Months		Right	Left	
Roentgen irradiation 200 r × 10	154	1	$\frac{9}{30}$	32	45	
	155	»	»	63	10	
	156	2	$1\frac{26}{30}$	4	6	
	157	4	$3\frac{11}{30}$	6	4	
	158	$5\frac{21}{30}$	$4\frac{28}{30}$	1	1	
	160	$6\frac{21}{30}$	$5\frac{29}{30}$	3	2	
	161	»	»	4	3	
Roentgen irradiation 400 r × 10	164	1	$\frac{9}{30}$	34	6	
	165	2	1	9	4	
	166	»	»	3	1	
	167	»	»	2	3	
	168	»	»	2	2	
	178	$19\frac{12}{30}$	$18\frac{18}{30}$	0	0	inflamed orbital glands
	179	»	»	1	2	
	177	$21\frac{24}{30}$	21	1	1	
	182	$22\frac{12}{30}$	$21\frac{18}{30}$	1	1	decapitated 26 hours after 0.2 mg colchicine
	183	»	»	3	1	

ROENTGEN IRRADIATION WITH 400 R FOR 10 CONSECUTIVE DAYS

For this experiment 10 rats were used. At the age of 1 month a clear deviation to the right of the nuclear class curve was observed (fig. 2 a). Four rats were killed at the age of 2 months and in these the difference between the nuclear class curve of the right irradiated gland and the left gland was still more pronounced (fig. 2 b). K_4 was the dominant class, K_2 being still the dominant class in the left gland.

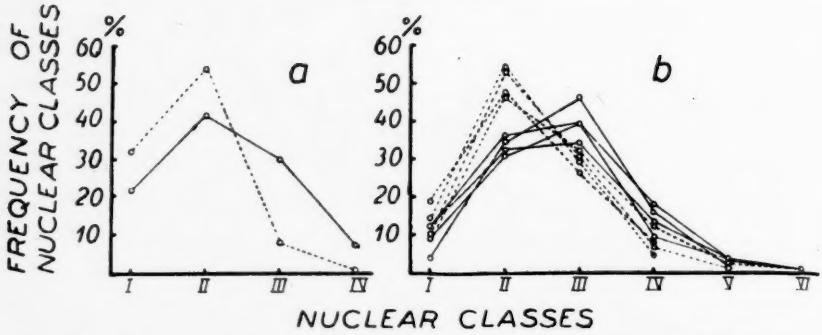

Fig. 2. — Diagram showing the nuclear class ratio in the outer orbital glands of rats irradiated 400 r × 10; a = at 1 month and b = at 2 months.

By means of the projection method (8) a control investigation was made on one rat in this group; the diameter of the nucleus was measured and the nuclear volume determined. If the results of nuclear class determination by the same method on normal rats are included, the nuclear class ratio may be characterized by the following values:

Nuclear classes:	*Irradiated:*	*Non-irradiated:*
First:	8 %; $K_1=244$	24 %; K_1 231
Second:	28 %; $K_2=508$, $2K_1=480$ (488)	55 %; $K_2=488$, $2K_1=451$ (462)
Third:	32 %; $K_4=991$, $2K_2=967$ (976)	20 %; $K_4=921$, $2K_2=879$ (924)
Fourth:	18 %; $K_8=2000$, $2K_4=1953$ (1952)	1 %; $K_8=1728$ (1948)
Fifth:	12 %; $K_{16}=3896$, $2K_8=3944$ (3904)	0 %
Sixth:	1 %; $K_{32}=7552$ (7808)	0 %
Seventh:	1 %; no clear maximum	0 %

This method, too, reveals a clear deviation in the percentile distribution of the nuclear classes after roentgen irradiation, and the result corresponds fairly well with the deviation obtained by the identification method (6) used in the present investigation. The average volume of the nuclear classes also corresponds fairly well with the theoretical value (stated in brackets) for the nuclear class in question.

In three animals, of which two were killed at the age of over 19 and one at the age of over 21 months, a slight chronic inflammation was noticed, similar to that earlier observed in this organ, and this has proved able in itself to cause deviation to the right of the

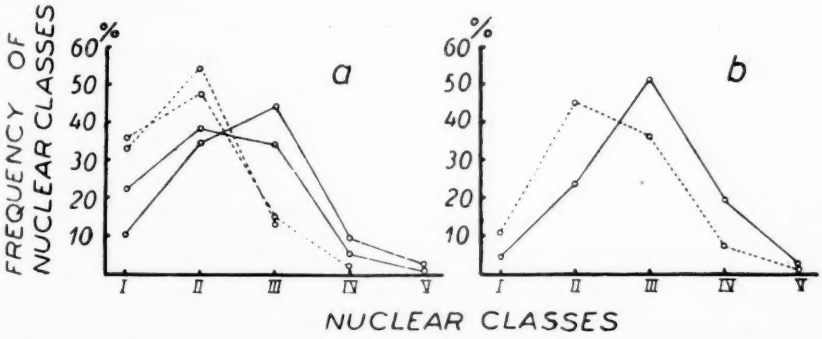

Fig. 3. — Diagram showing the nuclear class ratio after irradiation with 400 r × 10 in three animals with inflammation in the orbital glands; a = at 19 months, b = at 21 months.

nuclear class curve (6, 10). Although both orbital glands exhibited about the same degree of inflammation, a clear deviation to the right of the nuclear class curve in the right orbital gland could be established, as compared with the nuclear class curve of the left gland (fig. 3).

The two last rats in this group received 0.2 mg. of colchicine subcutaneously at an age of over 22 months and were decapitated 26 hours later. A deviation to the right of the nuclear class curve (fig. 4) similar to that in the earlier age groups in this experiment was also observed here in the right orbital gland.

In the rat that was killed 9 days after the last irradiation the mitotic ratio was higher in the right than in the left gland (table 1) Otherwise no relevant differences were observed between the two

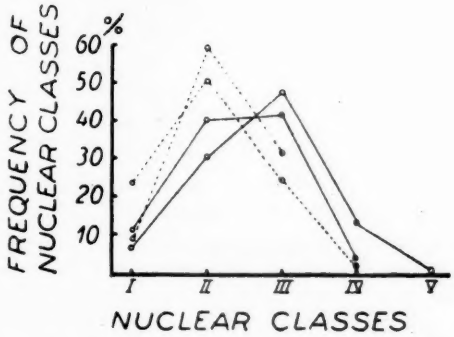

Fig. 4. — Diagram showing the nuclear class ratio in 22-month-old rats, irradiated with 400 r × 10; 26 hours after injection of 0.2 mg colchicine.

glands. In the inflamed glands (nos. 178, 179) isolated mitoses were found. It is remarkable that the two last rats in this group did not react to colchicine, although earlier experiments have given positive results (11) 5 months after single dose irradiation with 1000 r of 2-week-old rats. Since the experiment comprised only two animals no far-reaching conclusions can be drawn from the negative results.

DISCUSSION

The investigation showed that by means of roentgen irradiation with 2000 or 4000 r fractioned in equal doses of 200 and 400 r respectively during 10 consecutive days a clear deviation to the right of the nuclear class curve can be obtained in rats aged 12 days and over, more than 21 months after irradiation. This deviation to the right is in keeping with the change in the nuclear class curve achieved after single doses of 600, 1000, 1500 and 3000 r (11).

After a large dose the deviation took place earlier than after a small one. As was the case after the single dose irradiation, the change in the quantitative distribution of the nuclear classes was constant. By determination of the nuclear classes of an irradiated tissue one may therefore still, almost two years after the irradiation, register the effect of irradiation histoquantitatively.

From Helweg-Larsen's (3, 4) thorough investigations on the relation of the growth hormone of the anterior lobe of the hypophysis to nuclear class formation we know that this hormone is of the greatest importance in the control of the nuclear classes. There seems further to occur, in the epithelium of the uterine mucosa (5), a rhythmic nuclear class variation that follows the sexual cycle. A doubling of nuclear volume has further been produced experimentally in uterine gland cells by injection of oestrin into ovariectomized rats (1, 7). These experiments argue in favour of a superior humoral control of nuclear class formation and ratio. Roentgen irradiation of the orbital gland has shown, however, that at a point in the postembryonic growth period when only one and the lowest nuclear class is represented in the organ nuclear class determination can be so influenced that the otherwise comparatively constant cell number and comparatively constant ratio of the nuclear classes (8) can later be upset. By means of a locally effective outer factor it is

thus possible to produce growth by doubling in the same way as has been observed to happen in inflammatory changes of the organ (6, 10).

Such a deviation to the right of the nuclear class curve has been interpreted by some investigators (2) as a sign of malignancy. We were unable, however, to observe any indications of malignant degeneration in our rats. At the utmost one may here say that the irradiation energy influences the, according to Bizzozero's classification, stable orbital gland in such a way that mitoses are found after irradiation throughout life, just as is normally the case in labile organs.

The present investigation speaks for the correctness of the theory that cell growth and multiplication are controlled by a local system (9).

SUMMARY

1. By roentgen irradiation of the right outer orbital gland of rats aged 12 days for 10 consecutive days with 200 or 400 r, a clear deviation to the right of the nuclear class curve in this gland was obtained. The change was constant and could be observed till more than 22 months after irradiation.

2. By nuclear class determination one may still determine the irradiation effect histoquantitatively a very long time after irradiation.

3. In the irradiated gland a small number of mitoses were found very long after irradiation, although normally mitoses are only exceptionally seen in adult animals.

4. By means of roentgen irradiation one can obviously influence that system in the tissue which locally controls cell growth and multiplication there.

REFERENCES

1. Alfert, M., and Bern, H.: Proc. Nat. Acad. Sci. 1951:*37:*202.
2. Ehrich, W.: Zeitschr. f. Krebsforsch. 1936:*44:*308.
3. Helweg-Larsen, H.: Acta Pathol. et Microbiol. Scand. 1949:*26:*609.
4. Helweg-Larsen, H.: Nuclear Class Series, Munksgaard, Copenhagen. 1952.
5. Hintzsche, E.: Monatschr. f. Geburtsh. u. Gynäk. 1945:*120:*200, and Gynaecologia. 1949:*129:*1949.

6. JÄRVI, O., and TEIR, H.: Acta Pathol. et Microbiol. Scand. 1951:*29:*401.
7. SALVATORE, C. A.: Biol. Bulletin. 1950:*99:*112.
8. TEIR, H.: Acta Pathol. et Microbiol. Scand. 1944. Suppl. 54.
9. TEIR, H.: Comment. Biol. Soc. Scient. Fenniae. 1951:*13:*1.
10. TEIR, H.: Comment. Biol. Soc. Scient. Fenniae. 1951:*13:*16.
11. TEIR, H.: Acta Pathol. et Microbiol. Scand. 1952. In press.

FROM THE FIRST MEDICAL CLINIC, UNIVERSITY OF HELSINKI

COCARBOXYLASE CONTENT OF HUMAN BLOOD

STUDIES WITH NORMAL SUBJECTS AND IN NEUROCIRCULATORY ASTHENIA, THYROTOXICOSIS, ADIPOSITY, AND SOME OTHER DISEASES

A PRELIMINARY REPORT

by

WILLIAM KERPPOLA and TEPPO VARTIO

(Received for publication October 21, 1952)

Cocarboxylase, the pyrophosphoric acid ester of aneurin, is a coenzyme of carbohydrate metabolism, which, among other things, catalyses the decomposition of puryvic acid. Since increasing attention has been paid in recent years to the intermediary disturbances of metabolism present in many diseases, it seemed advisable to try to find out whether cocarboxylase showed any changes in various diseases. So far, little attention has been paid to this question. Westenbrink and co-workers (6, 7) found that the cocarboxylase content of the blood was 9—13 μg per 100 ml in normal persons. Peters and Rossiter (4) recorded lowered cocarboxylase values in the tissues of rats to which thyroxin had been administered. Florijn and Strengers (3) found that cocarboxylase values of blood are lowered in women towards the end of pregnancy and elevated in the newborn child. In untreated diabetes, Siliprandi and Navazio (5) reported lowered cocarboxylase values, which rose to normal by insulin therapy.

MATERIAL, METHOD, AND RESULTS

The studies were carried out between April 16 and August 23, 1952. The whole series of normal cases was collected during this time.

The series studied consisted of 55 normal subjects 19—54 years of age, one 77 years and of 22 patients with neurocirculatory asthe-

TABLE 1

COCARBOXYLASE CONTENT OF BLOOD IN NORMAL SUBJECTS

No.	Age	Sex	Constitution	Cocarboxylase μg per 100 ml	No.	Age	Sex	Constitution	Cocarboxylase μg per 100 ml
1	26	f	no particular type	11.7	29	21	f	no particular type	10.1
2	26	f	—»—	11.5	30	21	f	asthenic	11.9
3	25	m	pyknic	12.4	31	24	f	no particular type	10.9
4	40	m	no particular type	10.6	32	22	m	pyknic	12.0
5	49	m	—»—	11.8	33	33	m	»	13.5
6	34	m	pyknic	12.9	34	54	m	no particular type	14.9
7	50	m	no particular type	11.2	35	34	m	asthenic	9.4
8	25	m	asthenic	12.6	36	24	f	pyknic	14.2
9	19	f	no particular type	12.9	37	21	f	no particular type	12.0
10	29	f	pyknic	13.6	38	23	f	—»—	13.8
11	34	f	asthenic	10.4	39	20	f	—»—	14.0
12	22	f	no particular type	11.7	40	32	f	pyknic	12.1
13	23	f	—»—	14.2	41	33	f	»	14.0
14	19	f	pyknic	13.9	42	23	f	no particular type	12.8
15	20	f	no particular type	11.9	43	26	f	—»—	13.1
16	40	f	—»—	13.3	44	32	m	—»—	14.8
17	23	f	asthenic	10.4	45	36	f	—»—	14.0
18	21	f	no particular type	10.2	46	29	f	—»—	13.2
19	30	f	—»—	12.2	47	54	f	—»—	13.0
20	21	f	—»—	9.5	48	46	f	—»—	12.8
21	26	f	pyknic	14.0	49	32	f	asthenic	9.7
22	21	f	asthenic	9.4	50	44	m	pyknic	14.5
23	22	f	no particular type	13.7	51	33	m	no particular type	13.2
24	21	f	—»—	10.6	52	46	f	—»—	14.6
25	21	f	—»—	10.8	53	26	m	—»—	14.0
26	29	f	—»—	9.6	54	21	m	—»—	12.9
27	20	f	—»—	14.2	55	77	f	—»—	10.1
28	21	f	—»—	11.2	Mean 12.3 ± 1.20 μg per 100 ml				

nia[1], 8 patients with thyrotoxicosis, 18 with adiposity — of whom 8 also had hypertonia and 4 mild diabetes —, and 39 patients with other diseases.

The cocarboxylase determinations were made from whole blood by the Warburg manometer method of Westenbrink and co-workers (7). Two determinations were made from each specimen,

[1] The diagnosis was based on antecedent history characteristic of this disease and on the general absence of clinical signs.

TABLE 2

COCARBOXYLASE CONTENT OF BLOOD IN NEUROCIRCULATORY ASTHENIA

No.	Age	Sex	Erythrocytes mill./cu.mm	Leukocytes per cu.mm	Constitution	Cocarboxylase μg per 100 ml
1	25	m	4,850	5600	athletic	11.0
2	49	f	—	—	»	5.5
3	38	f	4,050	6400	»	5.2
4	34	m	5,290	9400	»	9.3
5	22	m	4,720	6500	asthenic	8.7
6	24	f	4,840	6300	»	7.7
7	48	m	5,320	5300	athletic	9.1
8	35	m	5,570	8200	asthenic	8.1
9	22	m	4,740	4400	»	7.9
10	22	m	4,350	5400	athletic	6.5
11	51	m	—	—	»	11.5
12	51	m	4,520	6000	»	6.1
13	23	m	—	—	»	8.0
14	14	f	4,780	6300	»	5.0
15	16	m	—	—	»	9.3
16	32	f	4,150	4100	»	8.7
17	42	f	—	—	pyknic	6.0
18	29	m	—	—	athletic	9.2
19	49	f	4,680	7100	pyknic	9.5
20	21	m	—	—	asthenic	11.3
21	47	f	—	—	»	10.9
22	30	m	—	—	athletic	11.7
Mean 8.5 ± 1.36 μg per 100 ml						

and their mean was calculated. In order to check the accuracy of the method, three series of eight simultaneous determinations from the same blood were carried out, the standard deviations of which were found to be 1.61, 1.61, and 0.95 μg per 100 ml with a mean of 1.4 μg per 100 ml. Thus the probability that the mean of two determinations deviates from the theoretical cocarboxylase value more than 3 μg per 100 ml is approximately 0.3 per cent. In order to find out the daily variability of the method, determinations were made from the same blood, kept in a refrigerator, on several successive days, the results being studied by means of variance analysis. It was found that the standard deviation of the method as calculated from double determinations and the deviation of the means of determinations made on several successive days could be taken as

TABLE 3

COCARBOXYLASE CONTENT OF BLOOD IN THYROTOXICOSIS

No.	Age	Sex	Erythrocytes mill./cu.mm	Leukocytes per cu.mm	Cocarboxylase μg per 100 ml
1	41	f	4,090	3400	9.7
2	44	f	4,850	4400	11.5
3	17	f	4,370	4500	6.9
4	22	f	—	7200	8.2
5	30	f	3,930	5500	10.3
6	55	f	4,940	6400	9.4
7	37	m	—	8400	7.6
8	54	f	4.050	2800	10.8
Mean 9.3 ± 1.78 μg per 100 ml					

calculated approximate values of the same theoretical deviation. It is also worthy of note that the cocarboxylase level of the blood showed no signs of falling in spite of the fact that the specimen was kept in a refrigerator even five days.

Considering the fact that cocarboxylase is only present in blood corpuscles, the number of which is therefore of significance for the determination of cocarboxylase from whole blood (2, 6), the number of leukocytes and erythrocytes was counted simultaneously with the determinations. In case the blood counts were within normal limits, no definite dependence of the cocarboxylase level upon the number of blood corpuscles was noted. The results are shown in tables 1 to 4.

DISCUSSION

The tables show that the normal values of blood cocarboxylase ranged between 9.4 and 14.9 μg per 100 ml, with a mean of 12.3± 1.20 μg per 100 ml. This agrees with previous studies (6). The group of neurocirculatory asthenia showed lowered values, 5.2 to 11.7 μg per 100 ml, with a mean of 8.5±1.36 μg per 100 ml. In 12 of 22 persons the values were between 5.2 and 8.7 μg per 100 ml. Lowered values occurred in the thyrotoxicosis group, too, in which the values ranged from 6.9 to 11.5 μg per 100 ml, with a mean of 9.3±1.78 μg per 100 ml. Elevated values, 12.7 to 25.5 μg per 100 ml, with a mean of 16.8±4.22 μg per 100 ml, were encoun-

TABLE 4

COCARBOXYLASE CONTENT OF BLOOD IN ADIPOSITY
(ADIPOSITY + HYPERTONIA AND ADIPOSITY + HYPERTONIA + DIABETES)

No.	Age	Sex	Height cm	Weight kg	Erythrocytes mill./cu.mm	Leukocytes per cu.mm	Cocarboxylase μg per 100 ml
				Adiposity			
1	22	m	176	90	—	—	16.4
2	54	f	159	74	4,740	7700	13.0
3	47	f	160	77	5,170	11600	14.4
4	45	f	157	71	4,100	—	19.0
5	40	f	159	78	4,420	3900	15.3
6	32	f	160	80	4,870	7000	17.5
				Adiposity + hypertonia			
1	66	m	173	94	5,780	9400	20.0
2	73	f	160	75	5,440	6500	12.9
3	53	f	159	78	4,580	6900	18.0
4	60	f	161	80	4,070	8500	14.0
5	76	f	162	77	3,540	6000	25.5
6	77	f	162	70	2,740	4300	16.9
7	36	m	169	87	—	—	12.7
8	71	f	158	78	4,890	8600	14.4
				Adiposity + hypertonia + diabetes			
1	57	m	172	95	—	—	22.0
2	68	f	158	72	—	—	13.0
3	69	f	157	70	—	—	17.4
4	69	f	160	76	—	—	19.5
				Mean 16.8 ± 4.22 μg per 100 ml			

tered in the group of adiposity (whether of simple adiposity or with elevated blood pressure and mild diabetes).

The mathematical-statistical treatment of the results by Sukhatme's test showed that in every group the difference in the mean values between the pathological and normal cases was significant with a probability of more than 99.8 per cent.

Cocarboxylase determinations have been made in 39 cases of other diseases, too. Elevated values have been found in Cushing's syndrome, acromegaly, rheumatic fever, and chronic rheumatoid polyarthritis. Particularly high values have been obtained in myeloid leukemia, (e.g. 51.7 μg per 100 ml in one patient with

207000 leukocytes per cu.mm) and also in lymphatic leukemia, though not so high as in myeloid leukemia. Low values have been recorded in some cases of ulcer and carcinoma. So far, the series reported have been small. In the normal series, pyknics seem to have higher values than asthenics. Work on the occurrence of cocarboxylase in blood is being continued. The fact that in certain diseases the blood cocarboxylase level is lower than normal might be explained by assuming that either a true vitamin deficiency or a disturbance in vitamin phosphorylation is present. Supplementary studies now in progress, in which both the blood cocarboxylase and the free vitamin B_1 content of the blood are determined, will throw some further light on the problem. The high cocarboxylase levels of obese subjects, who often suffer from increased blood pressure or diabetes, suggest the possibility of B_1-hypervitaminosis in persons with a certain type of bodily constitution and in diseases characteristic of people with this constitution.

SUMMARY

The writers' work on blood cocarboxylase in normal and pathological cases has so far yielded values which are lower than normal in neurocirculatory asthenia and thyrotoxicosis and elevated values in adiposity (simple or with elevated blood pressure and diabetes).

REFERENCES

1. Fischer, R. A., and Yates, T.: Statistical Tables. — Oliver & Boyd, Edinburgh, 1948.
2. Florijn, E., and Smits, G.: Nature 1948:*162:*220.
3. Florijn, E., and Strengers, H.: Acta Physiol. Pharmacol. Neerl. 1951:*2:*100.
4. Peters, R. A., and Rossiter, R. J.: Biochem. J. 1939:*33:*1140.
5. Siliprandi, N., and Navazio, F.: Acta Med. Scand. 1952:*142:*147.
6. Westenbrink, H. G. K.: Bull. Schweiz. Akad. Med. Wissensch. 1948: *4:*116.
7. Westenbrink, H. G. K., Steyn Parvé, E. P., van der Linden, A. C., and van den Broek, W. A.: Ztschr. Vitaminforsch. 1943:*13:*218.